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Supplementary Materials for

Unlocking P(V): Reagents for chiral phosphorothioate synthesis

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This PDF file includes:

Materials and Methods
Supplementary Text
Figs. S1 to S4
Tables S1 to S5
References
NMR Spectra

Table of Contents

1

GENERAL EXPERIMENTAL **8**

SYNTHESIS OF PSI REAGENTS **8**

COMPOUND 3 **8**
X-RAY CRYSTAL STRUCTURE OF COMPOUND 3 (TABLE S1) 10
(+)-*CIS*-LIMONENE OXIDE (2) **11**
COMPOUND (–)-1 [(–)-Ψ] **11**
SMALL SCALE PREPARATION (<100 G) 11
LARGE SCALE PREPARATION (>100 G) 12
DETERMINATION OF ENANTIOMERIC EXCESS 13
DIFFERENTIAL SCANNING CALORIMETRY DATA 14
COMPOUND (+)-1 [(+)-Ψ] **15**
X-RAY CRYSTAL STRUCTURE OF COMPOUND (+)-1 (TABLE S2) 15
PICTORAL GUIDE **17**
SYNTHESIS OF COMPOUND 3 17
SYNTHESIS OF COMPOUND 1 19

STEREOCHEMICAL ANALYSIS OF PSI REAGENTS **21**

ASSIGNMENT OF ABSOLUTE STEREOCHEMISTRY (FIGURE S1) **21**
LOADING/COUPLING ORDER EXPERIMENT (FIGURE S2) **22**
HPLC TRACES OF LOADING/COUPLING ORDER EXPERIMENT (FIGURE S3) **23**
X-RAY CRYSTAL STRUCTURE OF LOADED AZT NUCLEOSIDE (TABLE S3) **24**

P(V) REAGENT PLATFORM **25**

LEAVING GROUPS AND EPOXIDES (FIGURE S4) **25**

GENERAL PROCEDURE 1 - SYNTHESIS OF 5'-O-PROTECTED NUCLEOSIDES **26**

COMPOUND SI-1 **26**
COMPOUND SI-2 **26**
COMPOUND SI-3 **27**
COMPOUND SI-4 **27**
COMPOUND SI-5 **28**
PICTORAL GUIDE **28**
SYNTHESIS OF COMPOUND SI-5 28

GENERAL PROCEDURE 2 - SYNTHESIS OF 3'-O-PROTECTED NUCLEOSIDES **30**

COMPOUND SI-6 **30**
COMPOUND SI-7 **30**
COMPOUND SI-8 **31**
COMPOUND SI-9 **31**
COMPOUND SI-10 **31**
PICTORAL GUIDE **32**

SYNTHESIS OF COMPOUND SI-9 32

GENERAL PROCEDURE 3 – SYNTHESIS OF NUCLEOSIDE MONOMER SUCCINATES 34

COMPOUND SI-11 34
COMPOUND SI-12 34

OPTIMIZATION OF LOADING REACTION 36

GENERAL REACTION SCHEME 36
HPLC ASSAY 36
STANDARD METHOD FOR ASSAY DEVELOPMENT 36
STANDARD METHOD FOR SAMPLING 36
OPTIMIZATION TABLE (TABLE S4) 37

GENERAL PROCEDURE 4 – SYNTHESIS OF LOADED NUCLEOSIDES 38

COMPOUND (S_P)-4 38
COMPOUND (R_P)-4 39
COMPOUND (S_P)-5 39
COMPOUND (R_P)-5 40
COMPOUND (S_P)-6 41
COMPOUND (R_P)-6 41
COMPOUND (S_P)-7 42
COMPOUND (R_P)-7 43
COMPOUND (R_P)-22 44
COMPOUND SI-(R_P)-13 44
COMPOUND SI-(S_P)-13 45
COMPOUND SI-(R_P)-14 46
COMPOUND SI-(R_P)-15 46
COMPOUND SI-(R_P)-16 47
COMPOUND SI-(R_P)-17 48
COMPOUND SI-(R_P)-18 48
PICTORAL GUIDE 49
GENERAL PROCEDURE 4 (HOMOGENEOUS REACTION) 49
GENERAL PROCEDURE 4 (HETEROGENEOUS REACTION) 50

OPTIMIZATION OF COUPLING REACTION 51

SOLVENT SCREEN 51
CRUDE ^{31}P NMR ANALYSIS 51
BASE SCREEN 52
CRUDE ^{31}P NMR ANALYSIS 52

GENERAL PROCEDURE 5 – SYNTHESIS OF COUPLED DINUCLEOTIDES 52

COMPOUND (R_P)-8 53
COMPOUND (S_P)-9 53
COMPOUND (S_P)-10 54
COMPOUND (S_P)-11 55
COMPOUND (R_P)-12 56

COMPOUND (S_P)-13 **56**
COMPOUND (R_P)-14 **57**
COMPOUND (S_P)-15 **58**
COMPOUND (S_P)-16 **59**
COMPOUND (R_P)-17 **60**
UPLC/HPLC METHODS (TABLE S5) **61**
UPLC/HPLC CHROMATOGRAMS OF COMPOUNDS 8-17 **63**
PICTORAL GUIDE **73**
GENERAL PROCEDURE 5 – COUPLING 73

GENERAL PROCEDURE 6 – MANUAL SOLID-PHASE DINUCLEOTIDE SYNTHESIS **75**

MATERIALS **75**
SARCOSINE–SUCCINATE LINKER **75**
GENERAL SOLID-PHASE LOADING OF Ψ-COMPOUNDS **76**
CLEAVAGE **76**

SYNTHESIS OF CYLIC DINUCLEOTIDES: **77**

COMPOUND (S_P, R_P)-18 **77**
HPLC TRACE OF COMPOUND (S_P, R_P)-18 78
COMPOUND (S_P, S_P)-18 **79**
HPLC TRACE OF COMPOUND (S_P, S_P)-18 79
COMPOUND (R_P, S_P)-18 **80**
HPLC TRACE OF COMPOUND (R_P, S_P)-18 80
COMPOUND SI-(S_P)-19 **81**
COMPOUND (S_P, R_P)-19 **81**
HPLC TRACE OF COMPOUND (S_P, R_P)-19 82
COMPOUND (S_P)-20 **83**
COMPOUND SI-(S_P)-20 **83**
COMPOUND SI-(S_P, S_P)-21 **84**
COMPOUND (S_P, R_P)-21 **84**
HPLC TRACE OF COMPOUND (S_P, R_P)-21 86
COMPOUND SI-(S_P)-22 **87**
COMPOUND SI-(R_P)-22 **87**
PICTORAL GUIDE **88**
CDN SYNTHESIS – TBS DEPROTECTION 88
CDN MACROCYCLIZATION 89

SYNTHESIS OF (R_P) AND (S_P)-DT STANDARDS **91**

COMPOUND SI-(R_P)-23 **91**
STEP OPERATION REAGENTS AND SOLVENTS PARAMETERS 91
COMPOUND SI-(S_P)-23 **92**
STEP OPERATION REAGENTS AND SOLVENTS PARAMETERS 92

AUTOMATED SOLID-PHASE OLIGONUCLEOTIDE SYNTHESIS **94**

COMPOUND-(S_P, S_P, S_P, S_P)-23 **94**
STEP OPERATION REAGENTS AND SOLVENTS PARAMETERS 94

LCMS ANALYSIS OF (S_P, S_P, S_P, S_P)-23 96
COMPOUND-(*ALL*-R_P)-24 **97**
STEP OPERATION REAGENTS AND SOLVENTS PARAMETERS 97
LCMS ANALYSIS OF (ALL-R_P)-24 98

MANUAL SOLID-PHASE OLIGONUCLEOTIDE SYNTHESIS **99**

MATERIALS 99
GENERAL SOLID-PHASE DEPROTECTION AND EFFICIENCY EVALUATION 99
GENERAL SOLID-PHASE LOADING OF Ψ-COMPOUNDS 100
GENERAL SOLID-PHASE CAPPING 100
CLEAVAGE 100
PICTORIAL GUIDE **101**
COMPOUND SI-(*S*$_P$, *S*$_P$, *S*$_P$, *S*$_P$)-24 **102**

TROUBLESHOOTING AND FREQUENTLY ASKED QUESTIONS **103**

REAGENT SYNTHESIS. **103**
LOADING/COUPLING REACTIONS. **103**
SOLID-PHASE SYNTHESIS. **104**

NMR SPECTRA **105**

COMPOUND (–)-1 ^{1}H NMR **105**
COMPOUND (–)-1 ^{13}C NMR **106**
COMPOUND (–)-1 ^{31}P NMR **107**
COMPOUND (–)-1 ^{19}F NMR **108**
COMPOUND 3 ^{1}H NMR **109**
COMPOUND 3 ^{13}C NMR **110**
COMPOUND 3 ^{31}P NMR **111**
COMPOUND 3 ^{19}F NMR **112**
COMPOUND (*S*$_P$)-4 ^{1}H NMR **113**
COMPOUND (*S*$_P$)-4 ^{13}C NMR **114**
COMPOUND (*S*$_P$)-4 ^{31}P NMR **115**
COMPOUND (*R*$_P$)-4 ^{1}H NMR **116**
COMPOUND (*R*$_P$)-4 ^{13}C NMR **117**
COMPOUND (*R*$_P$)-4 ^{31}P NMR **118**
COMPOUND (*S*$_P$)-5 ^{1}H NMR **119**
COMPOUND (*S*$_P$)-5 ^{13}C NMR **120**
COMPOUND (*S*$_P$)-5 ^{31}P NMR **121**
COMPOUND (*R*$_P$)-5 ^{1}H NMR **122**
COMPOUND (*R*$_P$)-5 ^{13}C NMR **123**
COMPOUND (*R*$_P$)-5 ^{31}P NMR **124**
COMPOUND (*S*$_P$)-6 ^{1}H NMR **125**
COMPOUND (*S*$_P$)-6 ^{13}C NMR **126**
COMPOUND (*S*$_P$)-6 ^{31}P NMR **127**
COMPOUND (*R*$_P$)-6 ^{1}H NMR **128**
COMPOUND (*R*$_P$)-6 ^{13}C NMR **129**
COMPOUND (*R*$_P$)-6 ^{31}P NMR **130**
COMPOUND (*S*$_P$)-7 ^{1}H NMR **131**

COMPOUND (S_P)-7 ^{13}C NMR 132
COMPOUND (S_P)-7 ^{31}P NMR 133
COMPOUND (R_P)-7 ^{1}H NMR 134
COMPOUND (R_P)-7 ^{13}C NMR 135
COMPOUND (R_P)-7 ^{31}P NMR 136
COMPOUND (R_P)-8 ^{1}H NMR 137
COMPOUND (R_P)-8 ^{13}C NMR 138
COMPOUND (R_P)-8 ^{31}P NMR 139
COMPOUND (S_P)-9 ^{1}H NMR 140
COMPOUND (S_P)-9 ^{13}C NMR 141
COMPOUND (S_P)-9 ^{31}P NMR 142
COMPOUND (S_P)-10 ^{1}H NMR 143
COMPOUND (S_P)-10 ^{13}C NMR 145
COMPOUND (S_P)-10 ^{31}P NMR 146
COMPOUND (S_P)-11 ^{1}H NMR 147
COMPOUND (S_P)-11 ^{13}C NMR 148
COMPOUND (S_P)-11 ^{31}P NMR 149
COMPOUND (R_P)-12 ^{1}H NMR 150
COMPOUND (R_P)-12 ^{13}C NMR 151
COMPOUND (R_P)-12 ^{31}P NMR 152
COMPOUND (S_P)-13 ^{1}H NMR 153
COMPOUND (S_P)-13 ^{13}C NMR 154
COMPOUND (S_P)-13 ^{31}P NMR 155
COMPOUND (R_P)-14 ^{1}H NMR 156
COMPOUND (R_P)-14 ^{13}C NMR 157
COMPOUND (R_P)-14 ^{31}P NMR 158
COMPOUND (S_P)-15 ^{1}H NMR 159
COMPOUND (S_P)-15 ^{13}C NMR 160
COMPOUND (S_P)-15 ^{31}P NMR 161
COMPOUND (S_P)-16 ^{1}H NMR 162
COMPOUND (S_P)-16 ^{13}C NMR 163
COMPOUND (S_P)-16 ^{31}P NMR 164
COMPOUND (R_P)-17 ^{1}H NMR 165
COMPOUND (R_P)-17 ^{13}C NMR 166
COMPOUND (R_P)-17 ^{31}P NMR 167
COMPOUND (S_P, R_P)-18 ^{31}P NMR 168
COMPOUND (S_P, R_P)-18 ^{19}F NMR 169
COMPOUND (S_P, S_P)-18 ^{31}P NMR 170
COMPOUND (S_P, S_P)-18 ^{19}F NMR 171
COMPOUND (R_P, S_P)-18 ^{31}P NMR 172
COMPOUND (R_P, S_P)-18 ^{19}F NMR 173
COMPOUND (R_P, S_P)-19 ^{31}P NMR 174
COMPOUND (S_P, R_P)-19 ^{19}F NMR 175
COMPOUND (S_P)-20 ^{31}P NMR 176
COMPOUND (S_P, R_P)-21 ^{31}P NMR 177
COMPOUND (R_P)-22 ^{1}H NMR 178
COMPOUND (R_P)-22 ^{13}C NMR 179
COMPOUND (R_P)-22 ^{31}P NMR 180

COMPOUND SI-2 ^{1}H NMR **181**
COMPOUND SI-2 ^{13}C NMR **182**
COMPOUND SI-10 ^{1}H NMR **183**
COMPOUND SI-10 ^{13}C NMR **184**
COMPOUND SI-(R_P)-13 ^{1}H NMR **185**
COMPOUND SI-(R_P)-13 ^{13}C NMR **186**
COMPOUND SI-(R_P)-13 ^{31}P NMR **187**
COMPOUND SI-(R_P)-13 ^{19}F NMR **188**
COMPOUND SI-(S_P)-13 ^{1}H NMR **189**
COMPOUND SI-(S_P)-13 ^{13}C NMR **190**
COMPOUND SI-(S_P)-13 ^{31}P NMR **191**
COMPOUND SI-(S_P)-13 ^{19}F NMR **192**
COMPOUND SI-(R_P)-14 ^{1}H NMR **193**
COMPOUND SI-(R_P)-14 ^{13}C NMR **194**
COMPOUND SI-(R_P)-14 ^{31}P NMR **195**
COMPOUND SI-(R_P)-15 ^{1}H NMR **196**
COMPOUND SI-(R_P)-15 ^{13}C NMR **197**
COMPOUND SI-(R_P)-15 ^{31}P NMR **198**
COMPOUND SI-(R_P)-16 ^{1}H NMR **199**
COMPOUND SI-(R_P)-16 ^{13}C NMR **200**
COMPOUND SI-(R_P)-16 ^{31}P NMR **201**
COMPOUND SI-(R_P)-17 ^{1}H NMR **202**
COMPOUND SI-(R_P)-17 ^{13}C NMR **203**
COMPOUND SI-(R_P)-17 ^{31}P NMR **204**
COMPOUND SI-(R_P)-18 ^{1}H NMR **205**
COMPOUND SI-(R_P)-18 ^{13}C NMR **206**
COMPOUND SI-(R_P)-18 ^{31}P NMR **207**
COMPOUND SI-(S_P)-19 ^{31}P NMR **208**
COMPOUND SI-(S_P)-19 ^{19}F NMR **209**
COMPOUND SI-(S_P)-20 ^{31}P NMR **210**
COMPOUND SI-(S_P)-22 ^{31}P NMR **211**
COMPOUND SI-(S_P)-22 ^{19}F NMR **212**
COMPOUND SI-(R_P)-22 ^{31}P NMR **213**
COMPOUND SI-(R_P)-22 ^{19}F NMR **214**
COMPOUND SI-(S_P)-23 ^{31}P NMR **215**
COMPOUND SI-(R_P)-23 ^{31}P NMR **216**

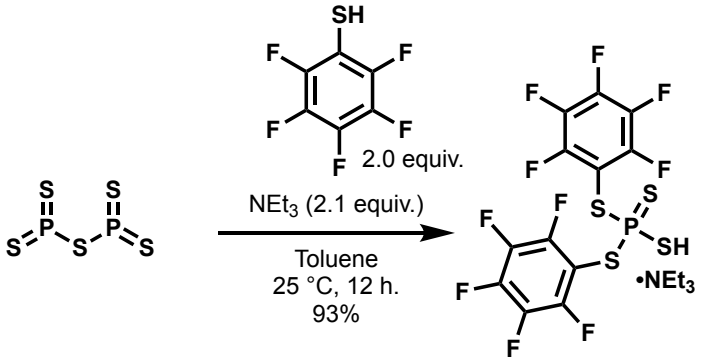
REFERENCES **217**

General Experimental

Tetrahydrofuran (THF), *N,N*-dimethylformamide (DMF), dichloromethane (DCM) and acetonitrile (MeCN) were obtained by passing the previously degassed solvents through an activated alumina column. All reagents were purchased at the highest commercial quality and used without further purification unless otherwise stated. Yields refer to chromatographically and spectroscopically (^{1}H NMR) homogenous material, unless otherwise stated. Reactions were monitored by thin-layer chromatography (TLC) or SHPLC. TLC was performed using 0.25 mm E. Merck silica plates (60F-254), using short-wave UV light as the visualizing agent, and phosphomolybdic acid, *p*-anisaldehyde, or $KMnO_4$ and heat as developing agents. Reactions were monitored by RP-HPLC analysis, see respective sections for details. NMR spectra were recorded on Bruker DRX-600, DRX-500, and AMX-400 instruments and were calibrated using residual undeuterated solvent (e.g., $CHCl_3$ at 7.26 ppm ^{1}H NMR, 77.16 ^{13}C NMR). The following abbreviations were used to explain multiplicities: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad. Column chromatography was performed using E. Merck silica gel (60, particle size 0.043–0.063 mm), and preparative TLC was performed on Merck silica plates (60F-254). High-resolution mass spectra (HRMS) were recorded on an Agilent LC/MSD TOF mass spectrometer by electrospray ionization time of flight reflectron experiments.

Synthesis of PSI Reagents

Compound 3



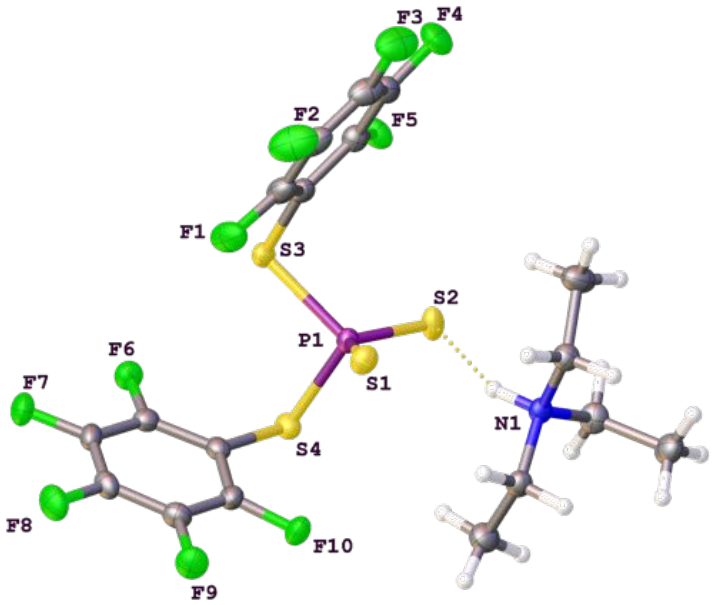
3

To a mixture of phosphorus pentasulfide (30.0 g, 132 mmol, 1.0 equiv.) in toluene (240 mL, 8 mL/g) was added pentafluorothiophenol (55.0 g, 267 mmol, 2.0 equiv.). The batch was made inert by flushing with nitrogen for 2 minutes. Triethylamine (39.0 mL, 277 mmol, 2.1 equiv.) was added over a period of 30 minutes (Note: The batch temperature reached 45 °C at the end of addition; the solution was allowed to reach ambient temperature by air cooling over an additional 30 minutes). The solution turned opaque yellow on addition of trimethylamine, then became cloudy. The mixture was stirred at ambient temperature overnight (Note: HPLC analysis at 3 hours indicated that RAP of pentafluorothiophenol vs. product <5%). The resulting slurry was filtered and the reaction vessel rinsed with toluene (2 x 30 mL); the rinses were flushed through the filter cake. The combined filtrates were concentrated under vacuum to 105 g (~3.5v). Methanol (180 mL, 6v) was added, followed by heptane (180 mL, 6v). The biphasic mixture was stirred for 15 minutes. Water (150 mL, 5v) was added over a period of 30 minutes. After the addition of water was

complete, the batch was mixed for 1 hour, then filtered. The reactor was washed with water/methanol (3:2 v/v, 75 mL) and the rinse was through the filter cake. The filter cake was washed with water (2 x 90 mL) followed by heptane (2 x 45 mL). The filter cake was dried *in vacuo* for 15 hours at 50 °C. **3** was isolated as a white crystalline solid (73.0 g, 93% yield).

Physical State: White crystalline solid;
^{1}H NMR (600 MHz, Chloroform-*d*): δ 8.57 (s, 1H), 3.27 (qd, *J* = 7.2, 4.5 Hz, 6H), 1.41 (t, *J* = 7.4 Hz, 9H);
^{13}C NMR (151 MHz, Chloroform-*d*): δ 148.87, 148.81, 148.78, 147.21, 147.15, 143.07, 142.99, 142.92, 141.37, 141.30, 141.22, 138.50, 136.94, 136.83, 136.73, 109.38, 46.74, 8.72;
^{19}F NMR (376 MHz, Chloroform-*d*): δ -129.17 (d, *J* = 21.6 Hz), -151.32 (td, *J* = 22.0, 21.1, 6.6 Hz), -162.07 (t, *J* = 20.8 Hz);
^{31}P NMR (162 MHz, Chloroform-*d*): δ 99.47;
mp: 75–78 °C.

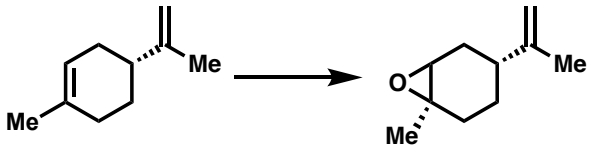
X-ray crystal structure of Compound 3 (Table S1)



Identification code	KK-03-720-9	
Empirical formula	C18 H16 F10 N P S4	
Formula weight	595.53	
Temperature	100.0 K	
Wavelength	1.54178 Å	
Crystal system	Triclinic	
Space group	P-1	
Unit cell dimensions	a = 7.8738(4) Å	α= 89.222(3)°.
	b = 8.5794(4) Å	β= 83.073(2)°.
	c = 17.9233(8) Å	γ = 88.966(2)°.
Volume	1201.65(10) $Å^3$	
Z	2	
Density (calculated)	1.646 Mg/m^3	
Absorption coefficient	5.083 mm^{-1}	
F(000)	600	
Crystal size	0.29 x 0.28 x 0.26 mm^3	
Theta range for data collection	2.483 to 68.509°.	
Index ranges	-9<=h<=9, -10<=k<=10, -21<=l<=21	
Reflections collected	18365	
Independent reflections	4333 [R(int) = 0.0339]	
Completeness to theta = 67.679°	98.1 %	
Absorption correction	Semi-empirical from equivalents	
Max. and min. transmission	0.7531 and 0.5414	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	4333 / 0 / 310	
Goodness-of-fit on F^2	1.183	
Final R indices [I>2sigma(I)]	R1 = 0.0344, wR2 = 0.0874	
R indices (all data)	R1 = 0.0361, wR2 = 0.0885	
Extinction coefficient	n/a	
Largest diff. peak and hole	0.480 and -0.276 $e.Å^{-3}$	

Table S1: Crystal data and structure refinement for **Compound 3**.

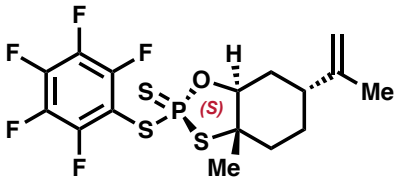
(+)-*cis*-Limonene oxide (2)



(+)-*cis*-limonene oxide (2)

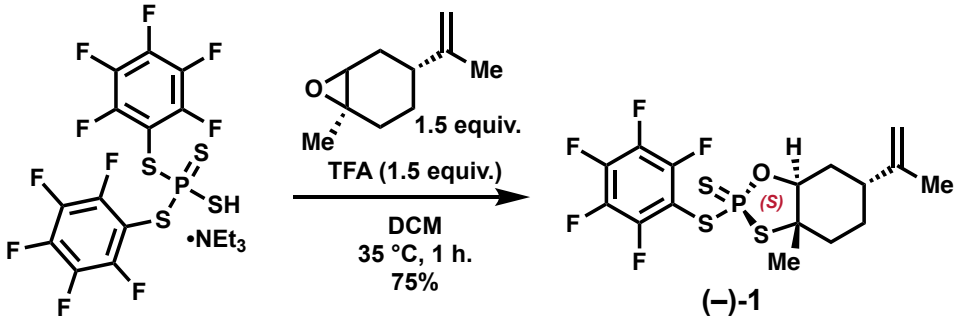
Compound (+)-**2** was prepared using a modified procedure (*41*, *42*): To a 500 mL Chemglass reactor was added 30% H_2O_2 (206 mL, 1.1 equiv.), phenylphosphonic acid (2.9 g, 0.01 equiv.), methyltrioctylammonium hydrogen sulfate (17.0 g, 0.02 equiv.), Na_2SO_4 (78.2 g, 0.30 equiv.), sodium tungstate decahydrate (12.1 g, 0.02 equiv.) followed by water (100 mL, 0.4 mL/g limonene). To the stirred solution was slowly added (+)-limonene (250 g, 1.0 equiv.) keeping the temperature below 30 °C. After stirring at 30 °C for 18 hours, the reaction was diluted with hexane (250 mL). The separated organic layer was washed with sodium bisulfite (100 mL, 10% aqueous), $NaHCO_3$ (100 mL, saturated aqueous), then brine (100 mL). The combined organic layers were dried over Na_2SO_4, filtered, then concentrated under reduced pressure. The crude limonene oxide was added to a 500 mL Chemglass reactor, followed by pyrollidine (153 mL, 1.0 equiv.) then water (26.4 mL, 0.80 equiv.). The reaction was stirred at 85 °C for 18 hours. The reaction was cooled to ambient temperature and hexane (250 mL) was added. The organics were washed with citric acid (20% aqueous) until <2% amino alcohol side product remained. The organics were washed with $NaHCO_3$ until pH >7 was obtained, followed by a brine wash. The compound was dried over Na_2SO_4, filtered, then concentration under reduced pressure. The crude compound was distilled (5 torr, 86–105 °C) to afford **2** (106 g, 38%) as a clear oil.

Compound (–)-1 [(–)-ψ]



(–)-1

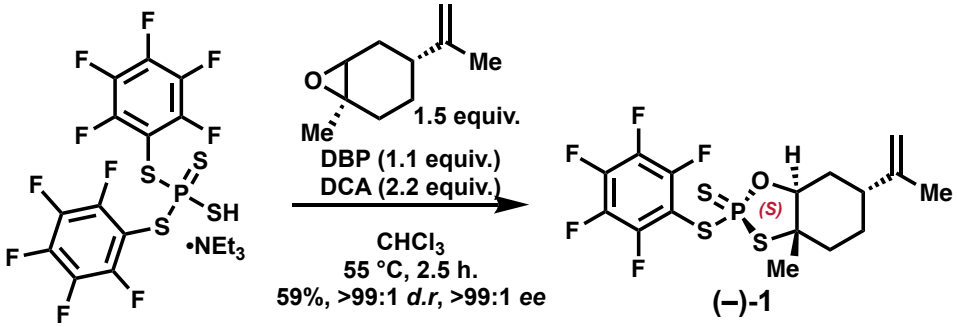
Small Scale Preparation (<100 g)



(–)-1

To a solution of **3** (1.00 g, 1.68 mmol, 1.0 equiv.) and cis-(+)-limonene oxide **2** (0.383 g, 2.52 mmol, 1.5 equiv.) in dichloromethane (5.0 mL, 5v) was added trifluoroacetic acid (0.19 mL, 2.52 mmol, 1.5 equiv.) The reaction was warmed to 35 °C and allowed to stir for 1 hour. The reaction mixture was cooled to ambient temperature, and hexanes (10 mL, 10 v) was added into the batch (Note: The resulting mixture was biphasic). The stream was washed with water (5 mL), saturated $NaHCO_3$ (10 mL), and KH_2PO_4 (10% aqueous, 3 mL). The organic phase was filtered through a $MgSO_4$ pad and concentrated to ~3 mL. Methanol (5 mL) was added, the batch was concentrated to ~3 mL; this procedure was repeated twice. The mixture was cooled to 5–10 °C and stirred for 5 min. The resulting slurry was filtered and the reactor and cake were washed with cold methanol (1 mL). The filter cake was dried *in vacuo* to afford **(–)-1 [(–)-ψ]** as a crystalline white solid (0.56 g, 75% yield).

Large Scale Preparation (>100 g)



To a solution of **3** (232 g, 390 mmol, 1.0 equiv.) and *cis*-(+)-limonene oxide **2** (90.0 g, 591 mmol, 1.5 equiv.) in chloroform (2.50 L, 0.156 M) was added dibutylphosphate (83.0 mL, 439 mmol, 1.1 equiv.) followed by dichloroacetic acid (72.0 mL, 872 mmol, 2.2 equiv.) (Note: The chloroform used contained 0.5-1% ethanol as a stabilizer). The reaction was warmed to 55 °C and allowed to stir for 2.5 hours. The reaction was concentrated to ~1.3 L under vacuum (200 torr, 35 °C). Hexane (2.0 L) was added and the combined organic layers were washed with K_2HPO_4 (10% aqueous, 1.5 L). The layers were separated and the combined organic layers washed with KH_2PO_4 (10% aqueous, 0.5 L) followed by water (0.5 L). The batch was distilled to 0.6 L in vacuo (200 torr, 35 °C). Methanol (0.75 L) was added and the batch was concentrated to 0.5 L as described above. Methanol (1.0 L) was added and the batch was concentrated to 1.5 L as described above. The resulting slurry was heated to 60 °C until complete dissolution of the solid reagents. The batch was cooled over 1 hour to 20 °C. Water (0.1 L) was added approximately half way through the cooling period. The batch was stirred for 15–24 hours at 20 °C. The resulting slurry was filtered and the filter cake was washed with water/methanol (1:9, 0.1 L) Note: The measured *d.r.* of the cake was 98:2 in this experiment. If the ratio is <20:1, dissolve the cake in MeOH (10 L/kg of cake) at 60 °C, cool the batch to 20 °C, add water (0.7 L/kg of cake), then mix the batch for 15–24 h.

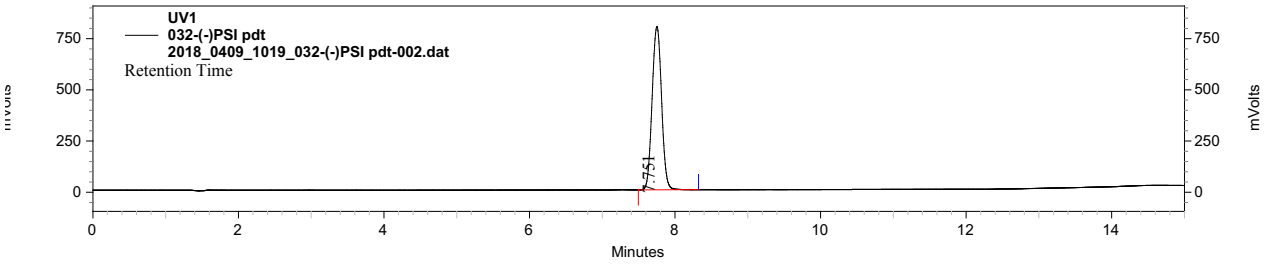
Recrystallization:

The cake (125 g) was dissolved in DCM (0.3 L) The batch was solvent-swapped to heptane, and distilled to 0.5 L. After mixing for 1 hour at 20 °C, the slurry was filtered, and the filtrate was recycled to complete the transfer of the slurry into the filter. The filter cake was washed with heptane (2 x 50 mL). After being dried at 50 °C under vacuum, **(–)-1 [(–)-ψ]** was isolated as a crystalline white solid (105 g, >99:1 *d.r.*; >99:1 *ee*, 59% yield).

Physical State: White crystaline solid;
^{1}H NMR (600 MHz, Chloroform-*d*): δ 5.02 (q, *J* = 1.5 Hz, 1H), 4.85 (d, *J* = 1.8 Hz, 1H), 4.27 (ddd, *J* = 12.7, 4.9, 3.6 Hz, 1H), 2.60 (d, *J* = 5.8 Hz, 1H), 2.37 – 2.31 (m, 1H), 2.07 – 1.85 (m, 4H), 1.84 – 1.79 (m, 3H), 1.80 – 1.68 (m, 1H), 1.66 (s, 3H).
^{13}C NMR (151 MHz, Chloroform-*d*): δ 148.23 (dt, *J* = 11.1, 4.2 Hz), 146.57 (dt, *J* = 11.2, 4.3 Hz), 144.49, 143.41 (dd, *J* = 13.5, 4.7 Hz), 142.10 – 141.37 (m), 138.24 (d, *J* = 17.0 Hz), 136.87 – 136.21 (m), 111.21, 105.10 – 103.97 (m), 86.14 (d, *J* = 3.2 Hz), 65.06, 38.43, 33.18 (d, *J* = 8.9 Hz), 27.21 (d, *J* = 14.7 Hz), 22.92, 22.03, 21.55.
^{19}F NMR (376 MHz, Chloroform-*d*): δ -130.02 (dd, *J* = 21.2, 4.6 Hz), -147.93 – -148.65 (m), -159.53 – -160.44 (m).
^{31}P NMR (162 MHz, Chloroform-*d*): δ 100.10.
$[\alpha]_D^{20}$ = -253 (*c* 0.5, DCM)
mp: 110 °C

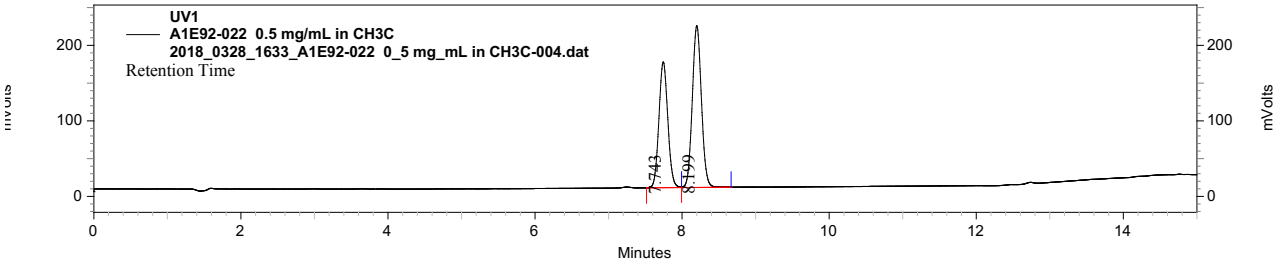
Determination of enantiomeric excess

Enantiomeric excess was determined using Chiralpak IB N-3 (150 x 4.6 mm. 3 uM) column eluting with 70 to 80% B (Mobile Phases: A: 5:95 MeCN/Water w/ 0.05% TFA, B: 95:5 MeCN,Water w/ 0.05% TFA) over 10 minutes, 1.2 mL/min, (–)-PSI: 7.75 min, (+)-PSI: 8.20 as (99% ee).



UV1 Results

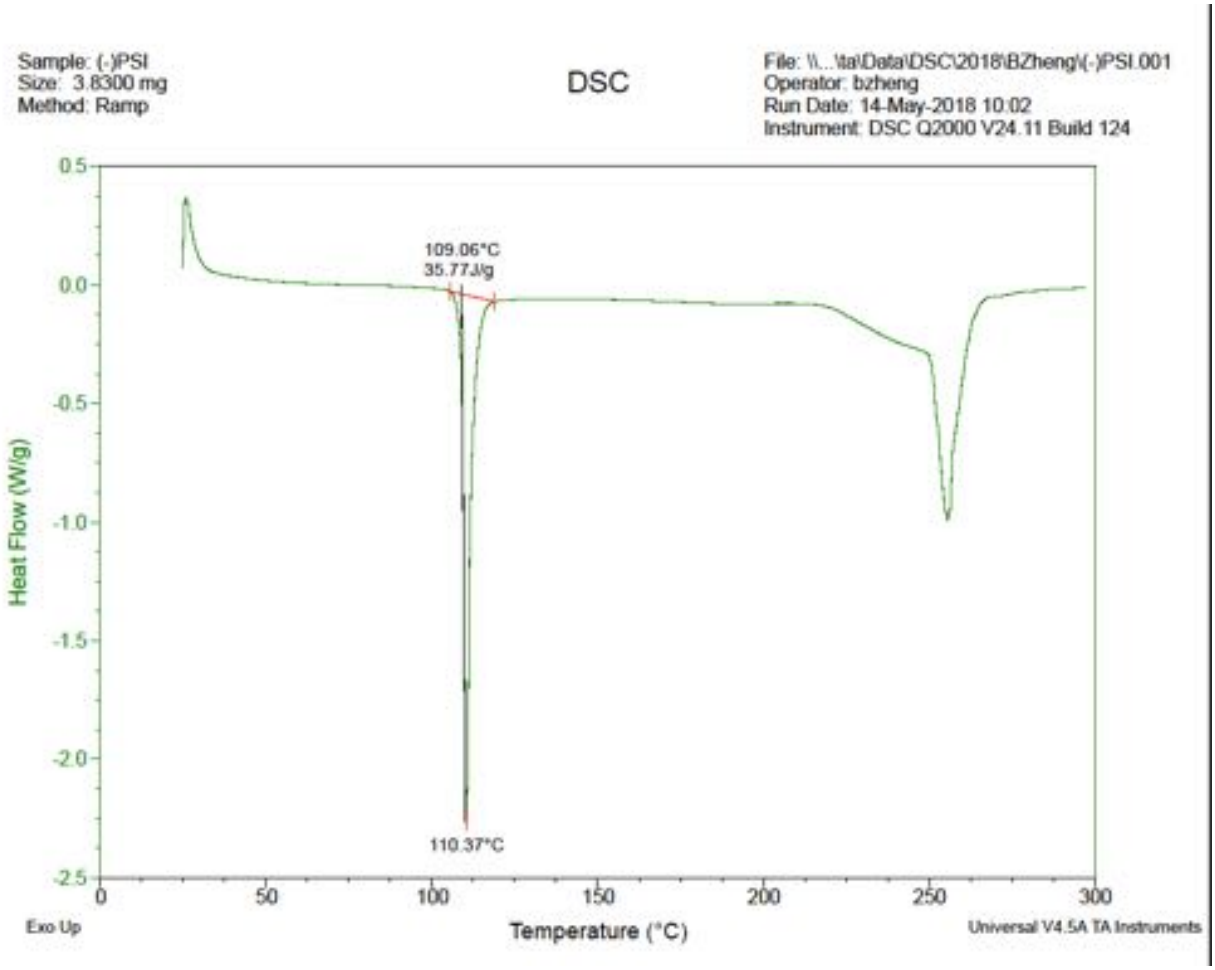
Pk #	RT (min)	Area	Area Percent	Name
1	7.75	7206714	100.00	(–)PSI



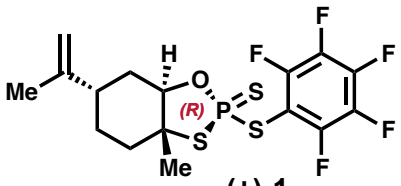
UV1 Results

Pk #	RT (min)	Area	Area Percent	Name
1	7.74	1401768	43.17	(–)PSI
2	8.20	1845390	56.83	(+)PSI

Differential scanning calorimetry data



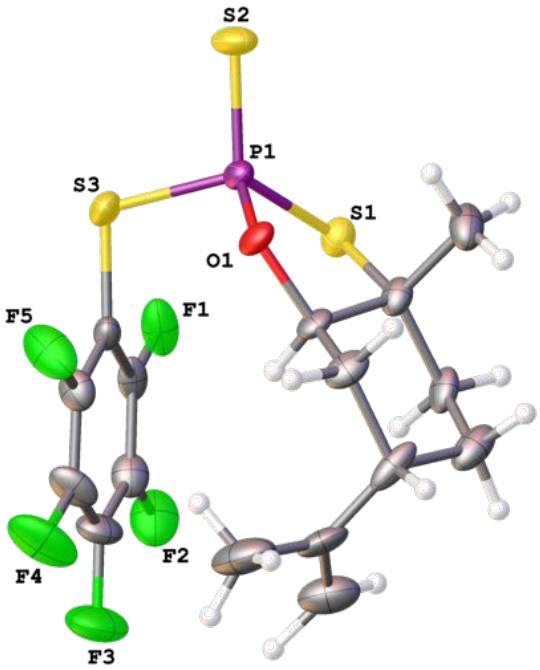
Compound (+)-1 [(+)-ψ]



(+)-1

Compound **(+)-1** prepared under identical conditions as **(–)-1** using **(–)-2**. All characterization data were identical, minus the optical rotation, $[\alpha]_D^{20} = 242$ (*c* 0.5, DCM).

X-ray crystal structure of Compound (+)-1 (Table S2)



Identification code	KK-03-574-9
Empirical formula	C16 H16 F5 O P S3
Formula weight	446.44
Temperature	100.0 K
Wavelength	0.71073 Å
Crystal system	Orthorhombic
Space group	$P2_12_12_1$
Unit cell dimensions	a = 9.5592(7) Å α= 90°.
b = 11.5870(9) Å	β= 90°.
c = 17.2072(9) Å	γ = 90°.
Volume	1905.9(2) $Å^3$
Z	4
Density (calculated)	1.556 Mg/m^3
Absorption coefficient	0.523 mm^{-1}
F(000)	912
Crystal size	0.3 x 0.27 x 0.23 mm^3
Theta range for data collection	2.119 to 25.345°.
Index ranges	-11<=h<=11, -8<=k<=13, -20<=l<=20
Reflections collected	11357

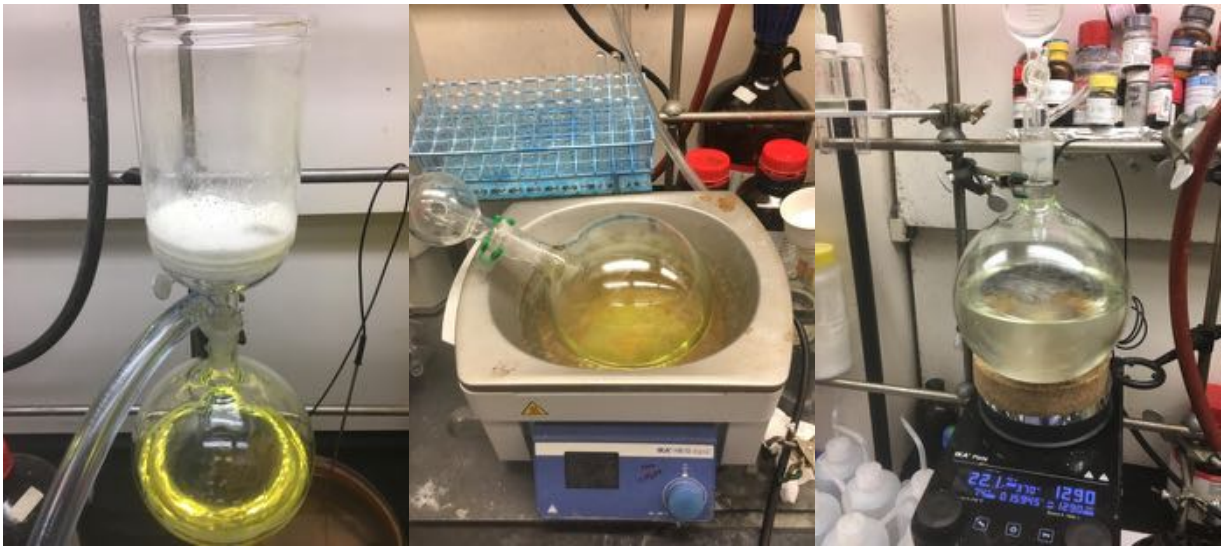
Independent reflections	3471 [R(int) = 0.0304]
Completeness to theta = 25.242°	100.0 %
Absorption correction	Semi-empirical from equivalents
Max. and min. transmission	0.7452 and 0.7084
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	3471 / 0 / 245
Goodness-of-fit on F^2	1.046
Final R indices [I>2sigma(I)]	R1 = 0.0382, wR2 = 0.0848
R indices (all data)	R1 = 0.0452, wR2 = 0.0886
Absolute structure parameter	0.09(4)
Extinction coefficient	n/a
Largest diff. peak and hole	0.478 and -0.302 e.$Å^{-3}$

Table S2: Crystal data and structure refinement for **Compound (+)-1**.

Pictoral Guide

Synthesis of Compound 3

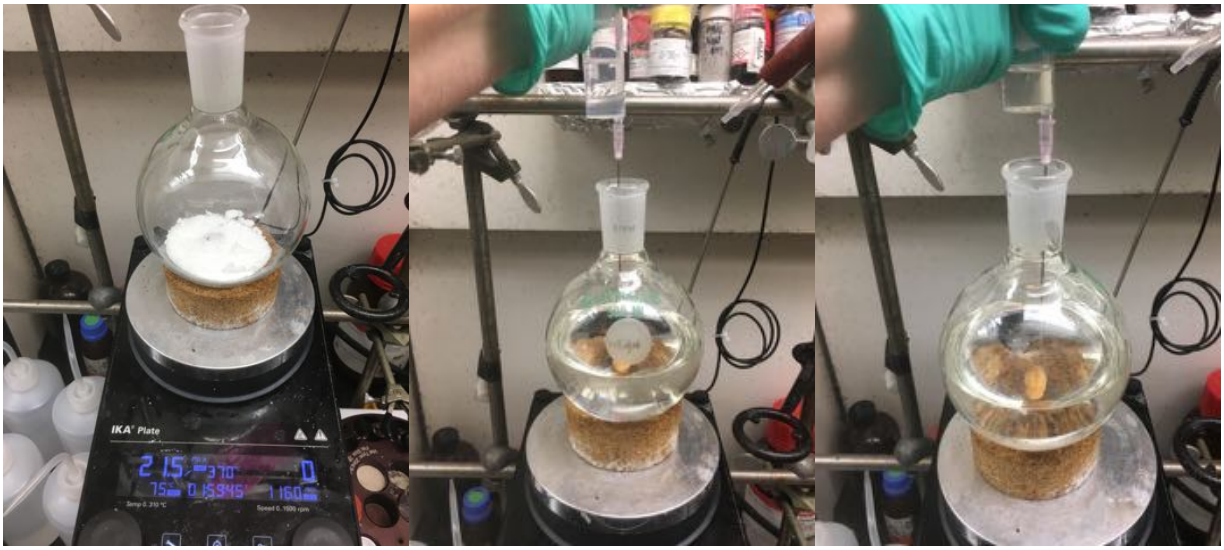
(From left to right): Toluene suspension of P_2S_5 and pentafluorothiophenol, dropwise addition of triethylamine, suspension formed after stirring overnight.



(From left to right): Filtration of suspension, concentration of filtrate, biphasic mixture formed after dilution of toluene solution with methanol and heptane.

(From left to right): Slurry formed during the slow addition of water, crystals of **3** collected by filtration.

Synthesis of Compound 1



(From left to right): Flask charged with **3**, after dissolving in $CHCl_3$ dibutylphosphate was added, addition of dichloroacetic acid.

(From left to right): 65 °C hold for 2.5 hours, reaction volume concentrated ~ ½ volume, dilution of the crude reaction with hexane.

(From left to right): Aqueous work up, crude reaction after aqueous work up, concentration during solvent swap to methanol.

(From left to right): Pure **1** isolated by filtration.

Stereochemical Analysis of PSI Reagents

Assignment of absolute stereochemistry (Figure S1)

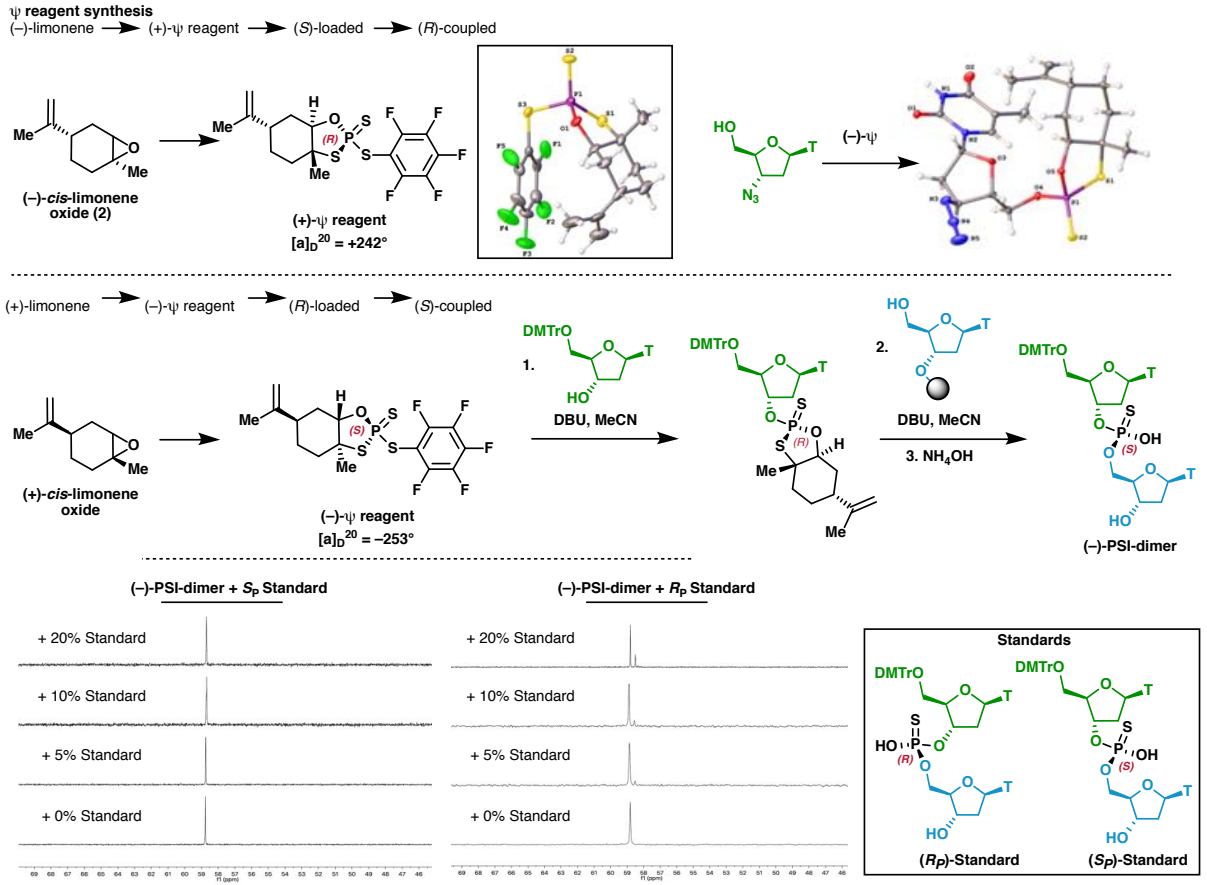


Figure S1: Assignment of absolute stereochemistry, comparing (–)-ψ nucleoside dimer with known (R_P) and (S_P) standards.

Loading/Coupling Order Experiment (Figure S2)

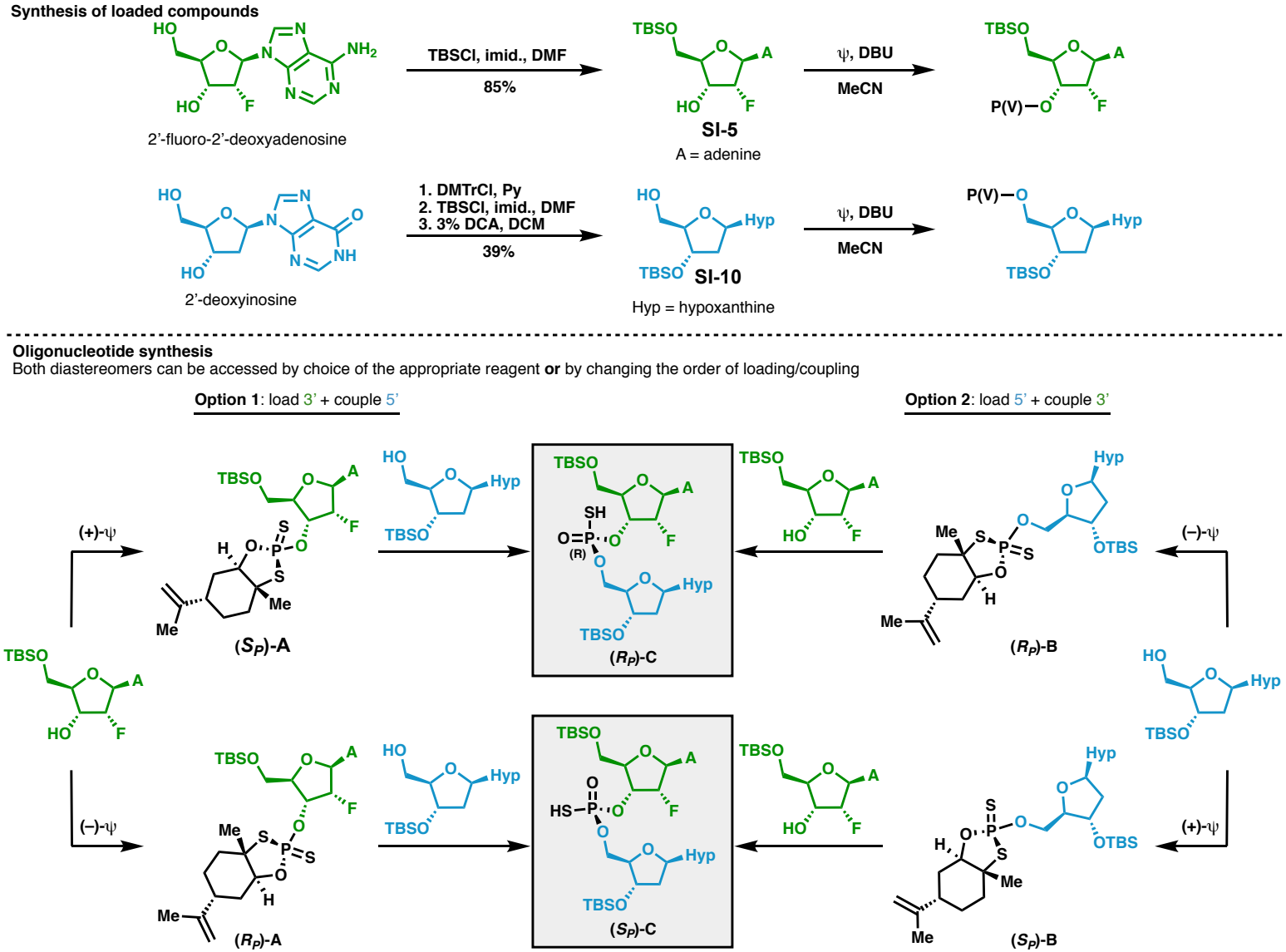


Figure S2: Absolute phosphorous stereochemistry is dictated by the order of loading and coupling steps.

HPLC Traces of Loading/Coupling order experiment (Figure S3)

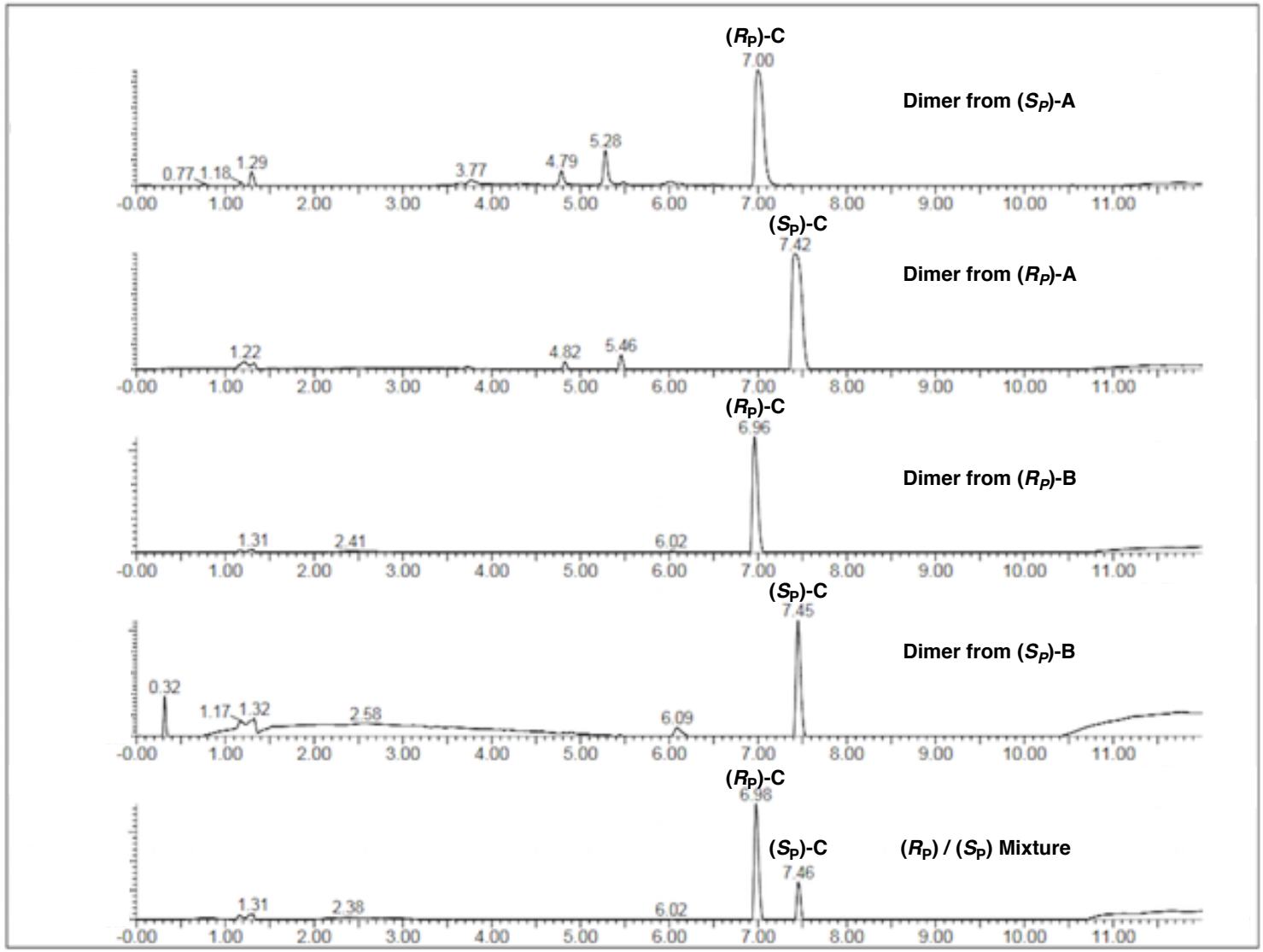
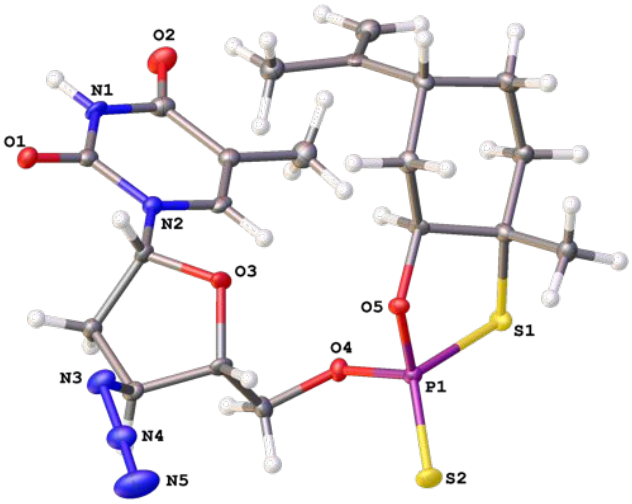


Figure S3: HPLC Traces of (R_P)-C and (S_P)-C dinucleotides.

X-ray crystal structure of loaded AZT nucleoside (Table S3)



Identification code	KK-03-720-01	
Empirical formula	C20 H28 N5 O5 P S2	
Formula weight	513.56	
Temperature	100.0 K	
Wavelength	0.71073 Å	
Crystal system	Orthorhombic	
Space group	$P2_12_12_1$	
Unit cell dimensions	a = 7.4416(3) Å	α= 90°.
	b = 10.5158(3) Å	β= 90°.
	c = 30.7194(12) Å	γ = 90°.
Volume	2403.93(15) $Å^3$	
Z	4	
Density (calculated)	1.419 Mg/m^3	
Absorption coefficient	0.330 mm^{-1}	
F(000)	1080	
Crystal size	0.33 x 0.31 x 0.29 mm^3	
Theta range for data collection	2.047 to 26.380°.	
Index ranges	-8<=h<=9, -12<=k<=13, -38<=l<=36	
Reflections collected	14556	
Independent reflections	4894 [R(int) = 0.0597]	
Completeness to theta = 25.242°	100.0 %	
Absorption correction	Semi-empirical from equivalents	
Max. and min. transmission	0.7454 and 0.6961	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	4894 / 0 / 309	
Goodness-of-fit on F^2	1.030	
Final R indices [I>2sigma(I)]	R1 = 0.0388, wR2 = 0.0853	
R indices (all data)	R1 = 0.0452, wR2 = 0.0889	
Absolute structure parameter	-0.05(6)	
Extinction coefficient	n/a	
Largest diff. peak and hole	0.248 and -0.276 e.$Å^{-3}$	

Table S3: Crystal data and structure refinement for loaded AZT nucleoside.

P(V) Reagent Platform

Leaving groups and epoxides (Figure S4)

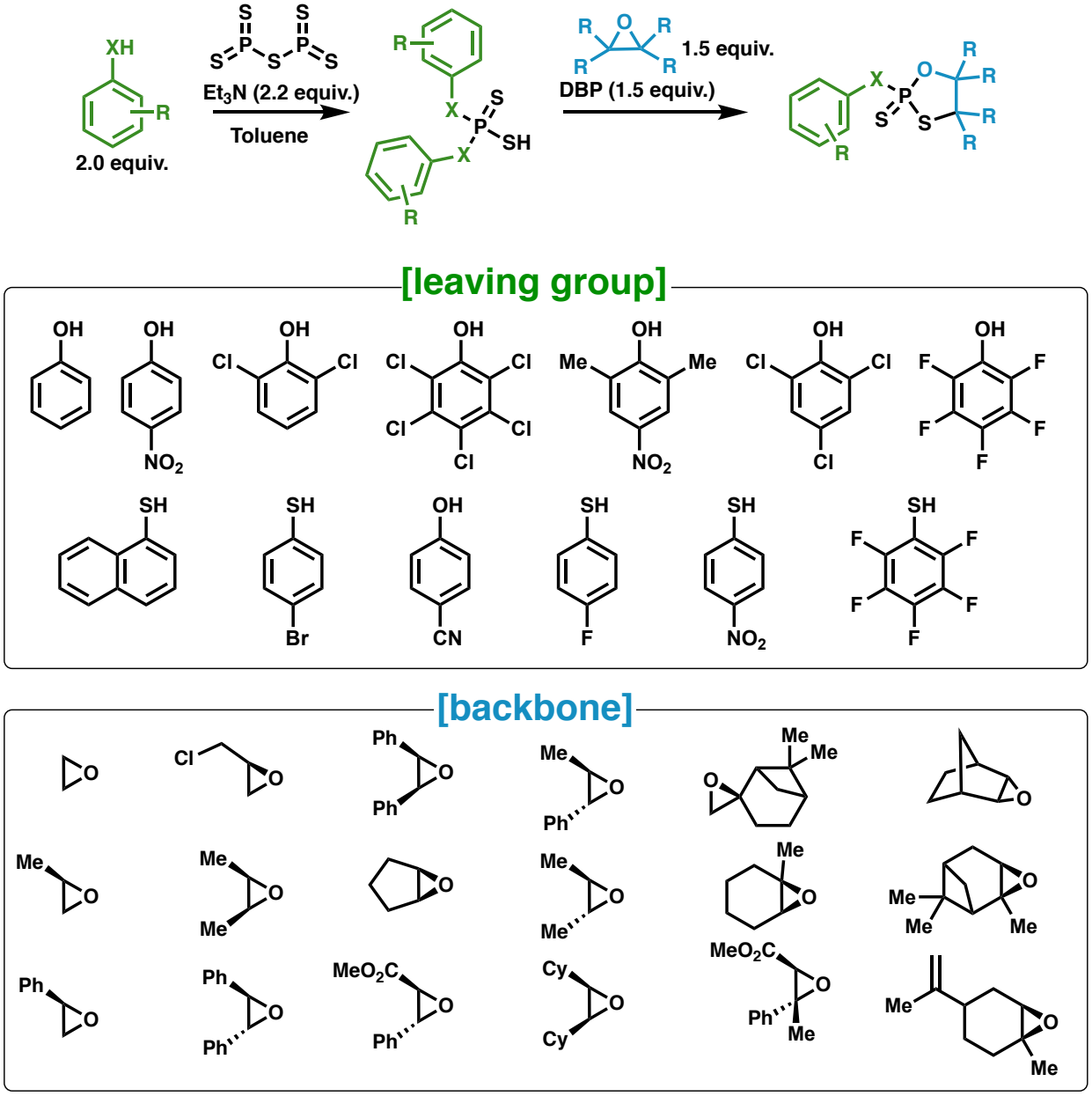
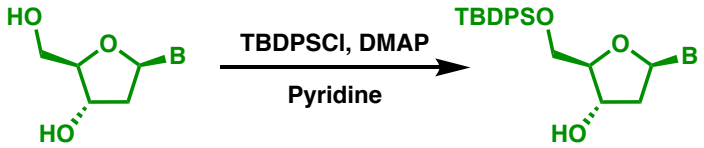


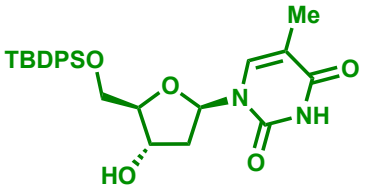
Figure S4. Selected scope of epoxides and leaving groups for generating P(V) reagents (additional details will be included in full account).

General Procedure 1 - Synthesis of 5'-O-Protected Nucleosides



All 5'-OTBDPS nucleosides were prepared from the commercially available compounds according to the following procedures.

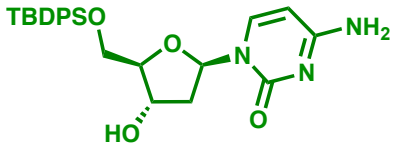
Compound SI-1



SI-1

To a solution of 2′-deoxythymidine (15.0 g, 61.3 mmol. 1.0 equiv.) and DMAP (1.51 g, 12.26 mmol, 0.2 equiv.) in dry pyridine (75 mL) was added TBDPSCl (18.7 mL, 70.5 mmol, 1.2 equiv.). The mixture was stirred for 16 h, then diluted with EtOAc (200 mL) and washed with water (200 mL), ammonium chloride (saturated aqueous, 100 mL), then brine (100 mL). The organic layer was dried over $MgSO_4$, filtered, then concentrated *in vacuo* to afford a residue that was purified by flash column chromatography over silica gel (25% EtOAc in hexanes to 100% EtOAc). Residual pyridine was removed by stirring in MTBE/hexanes (1:1, v/v, 150 mL). The resulting solid was isolated by filtration and washed with MTBE (100 mL). The white solid was dried at 50 °C and 20 torr until constant weight was achieved. **SI-1** (22.59 g, 77%) was isolated with spectral characteristics consistent with the literature (*43*).

Compound SI-2



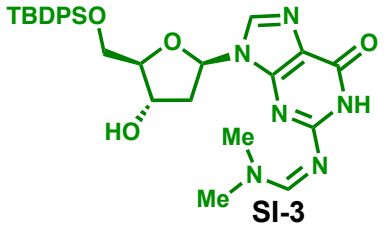
SI-2

SI-2 prepared from 2′-deoxycytidine (15.0 g) under the same protocol as **SI-1**. Following completion of the reaction as assessed by TLC, the mixture was concentrated *in vacuo*. The crude syrup was dissolved in EtOAc (500 mL), washed with 1 N HCl (150 mL, final pH = 6), washed with sodium phosphate (100 mL, 1.0 M, pH=7) buffer, then brine (200 mL). The organics were dried over $MgSO_4$, filtered, and concentrated to afford a thick oil. The oil was purified by flash column chromatography (0% to 10% MeOH in DCM). The isolated foam was broken down into MTBE/hexane (1:1 v/v, 200 mL) then filtered and the solid was dried at 50 °C at 20 torr until constant weight was achieved. **SI-2** (20.0 g, 66%) was isolated as a white solid.

Physical State: White solid;

^{1}H NMR (600 MHz, DMSO-d_6): δ 7.70 (d, J = 7.4 Hz, 1H), 7.64 (dq, J = 6.6, 1.2 Hz, 4H), 7.52 – 7.41 (m, 6H), 7.17 (s, 1H), 7.13 (s, 1H), 6.18 (t, J = 6.5 Hz, 1H), 5.56 (d, J = 7.4 Hz, 1H), 5.29 (d, J = 4.5 Hz, 1H), 4.29 (dq, J = 6.0, 3.9 Hz, 1H), 3.89 – 3.82 (m, 1H), 3.85 (s, 1H), 3.78 – 3.71 (m, 1H), 2.19 (ddd, J = 13.2, 6.3, 4.1 Hz, 1H), 1.99 (dt, J = 13.2, 6.5 Hz, 1H), 1.01 (s, 9H);
^{13}C NMR (151 MHz, DMSO-d_6): δ 165.26, 154.67, 140.49, 135.15, 134.99, 132.69, 132.38, 130.02, 129.98, 127.98, 93.83, 86.35, 84.68, 69.77, 63.82, 40.51, 26.84, 26.67, 18.81;
HRMS (ESI-TOF): calcd. for $C_{25}H_{32}N_3O_{8Si}$ $[M + H]^+$466.2156; found 466.2157.

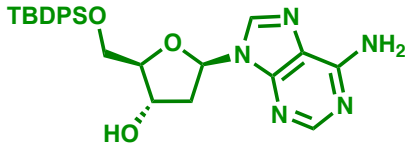
Compound SI-3



SI-3

2′-Deoxyguanosine was first converted to the dimethylformyl protected compound (*44*) to increase solubility in pyridine. To a solution of 2′-deoxyguanosine (30.0 g, 112 mmol, 1.0 equiv.) in methanol (300 mL) was added *N*,*N*-dimethylformamide dimethyl acetal (60 mL). After stirring for 72 h, the reaction was filtered and washed with MeOH (200 mL). To a solution of the DMF protected intermediate (34.4 g, 107 mmol, 1.0 equiv.) and DMAP (2.63 g, 21.4 mmol, 0.2 equiv.) in dry pyridine (344 mL) was added TBDPSCl (32.6 mL, 123 mmol, 1.2 equiv.). After stirring for 24 h the reaction was concentrated *in vacuo*. The residue obtained was partitioned between EtOAc (500 mL) and HCl (1 N, 500 mL, final pH=6). The organic layer was washed with brine (500 mL), dried over Na_2SO_4, filtered, then concentrated. The crude oil was purified by flash column chromatography (0% to 10% MeOH in DCM). The resulting solids obtained were stirred with MTBE for 1 h, filtered then dried to constant weight. The stir/filter protocol was repeated twice. **SI-3** (40.0g g, 67%) was isolated with spectral characteristics consistent with the literature (*45*).

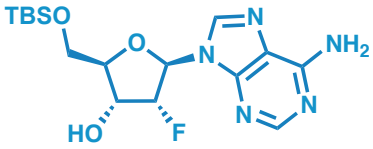
Compound SI-4



SI-4

2′-Deoxyadenosine (15.0 g, 58.5 mmol, 1.0 equiv.) and imidazole (9.96 g, 146 mmol, 2.5 equiv.) were dissolved in dry pyridine (100 mL) and concentrated *in vacuo*. The resulting mixture was dissolved in anhydrous DMF (75 mL), TBDPSCl (16.3 mL, 61.4 mmol, 1.1 equiv.) was added, and the mixture was stirred for 2 h. The solution was concentrated *in vacuo* then dissolved in EtOAc (500 mL), and washed with water (2 x 250 mL), then brine (250 mL). The organic layer was dried over $MgSO_4$, filtered, and concentrated *in vacuo* to afford a thick oil that was purified by flash column chromatography over silica gel (0% to 5% MeOH in EtOAc) to afford the product as a white solid. **SI-4** (18.0 g, 63%) was isolated with spectral characteristics consistent with the literature (*46*).

Compound SI-5



SI-5

2'-Deoxy-2'-fluoroadenosine (2.10 g, 7.81 mmol, 1.0 equiv.) was dried by co-evaporation with anhydrous pyridine (20 mL x 3) *in vacuo* then dissolved in anhydrous DMF (40 mL, 0.2 M) in a flame-dried round bottom flask. Imidazole (1.17 g, 17.2 mmol, 2.2 equiv.) was added, followed by TBSCl (1.29 g, 8.60 mmol, 1.1 equiv.). The reaction was stirred overnight at ambient temperature. The reaction was quenched on addition of water (40 mL). After stirring for 20 minutes, the solids were collected by filtration and washed with water (40 mL). After drying *in vacuo* overnight, **SI-5** (2.54 g, 85%) was isolated as a white solid with spectral characteristics consistent with the literature (*47*).

Pictoral Guide

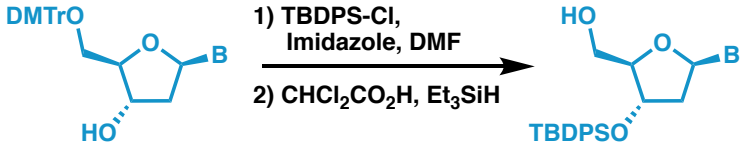
Synthesis of Compound SI-5

(From left to right): Pyridine and 2'-Deoxy-2'-fluoroadenosine, co-evaporation of 2'-Deoxy-2'-fluoroadenosine with anhydrous pyridine, dried 2'-Deoxy-2'-fluoroadenosine.

(From left to right): 2’-Deoxy-2’-fluoroadenosine diluted in DMF, 2’-Deoxy-2’-fluoroadenosine in DMF, imidazole and TBSCl, overnight stirring, water quench.

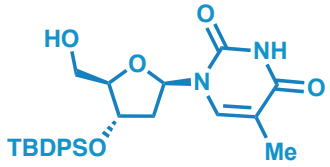
(From left to right): reaction mixture after quench, filtration of the desired TBS protected 2’-Deoxy-2’-fluoroadenosine.

General Procedure 2 - Synthesis of 3'-O-Protected Nucleosides



3'-OTBDPS protected nucleosides were prepared from the commercially available 5'-ODMTr nucleosides according to the following procedures.

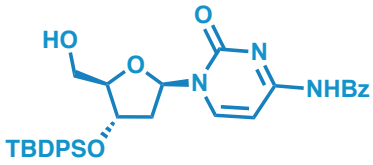
Compound SI-6



SI-6

To a solution of 5′-O-(4,4′-dimethoxytrityl)thymidine (15.0 g, 27.6 mmol, 1.0 equiv.) and imidazole (3.14 g, 46.2 mmol, 2.0 equiv.) in DMF (30 mL) was added TBDPS-Cl (7.39 g, 33.1 mmol, 1.2 equiv.) at ambient temperature and stirred for 3 days. The resulting mixture was then poured into water (0.7 L), and mixed for 0.5 h. The slurry was filtered and the cake was washed with water, and then hexanes. The filter cake was dissolved in DCM. The resulting solution washed with 5% aqueous citric acid solution and dried over $MgSO_4$. To the resulting DCM stream was added dichloroacetic acid (11.4 mL) and Et_3SiH (18 mL). After stirring for 18 h, the mixture was quench with saturated aqueous $NaHCO_3$ and heptane. The isolated organic layer was concentrated, and the resulting residue was purified by chromatography(EtOAc/DCM). **SI-6** (10.1g, 75%) was isolated as a white solid with spectral characteristics consistent with the literature (*48*).

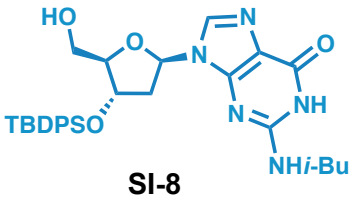
Compound SI-7



SI-7

Compound **SI-7** was prepared analogously to **SI-6** using N^4-benzoyl-5′-*O*-(4,4′-dimethoxytrityl)-2′-deoxycytidine (15.0 g, 23.7 mmol, 1.0 equiv.). **SI-7** (10.3 g, 73%) was isolated as a white solid with spectral characteristics consistent with the literature (*48*).

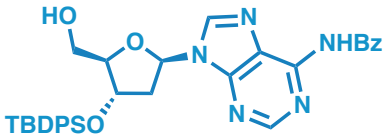
Compound SI-8



SI-8

Compound **SI-8** was prepared analogously to **SI-6** using N^2-isobutyryl-5′-O-(4,4′-dimethoxytrityl)-2′-deoxyguanosine (15.0 g, 23.5 mmol, 1.0 equiv.). **SI-8** (9.8 g, 75%) was isolated as a white solid with spectral characteristics consistent with the literature (*48*).

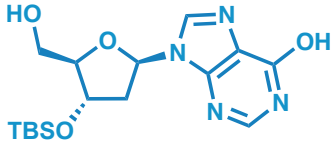
Compound SI-9



SI-9

Compound **SI-9** was prepared analogously to **SI-6** using N^6-benzoyl-5'-O-(4,4'-dimethoxytrityl)-2'-deoxyadenosine (35.0 g, 53.3 mmol, 1.0 equiv.). **SI-9** (17.8 g, 56%) was isolated as a white solid with spectral characteristics consistent with the literature (*48*).

Compound SI-10



SI-10

2'-deoxyinosine (4.40 g, 17.4 mmol, 1.0 equiv.) was dried by co-evaporation with anhydrous pyridine (20 mL x 3) *in vacuo* then dissolved in anhydrous pyridine (75 mL) in a flame-dried round-bottom flask. 4,4′-Dimethoxytrityl chloride (8.50 g, 25.1 mmol, 1.4 equiv.) was added as a solid in small portions over 10 minutes. The reaction was stirred at ambient temperature overnight then concentrated *in vacuo*, diluted in DCM (75 mL) and washed with sat. aq. $NaHCO_3$ (75 mL). The aqueous layer was separated and washed with DCM (75 mL x 3). The combined organic layers were washed with brine (75 mL), dried over $MgSO_4$ and the solvent was removed *in vacuo*. The crude residue and imidazole (2.60 g, 38.2 mmol, 2.2 equiv.) were dissolved in anhydrous DMF (25 mL). The solution was cooled to 0 °C then TBSCl (5.30 g, 35.2 mmol, 2.0 equiv.) was added in one portion and the reaction was stirred at ambient temperature for 24 h. Partial conversion was observed by TLC analysis. A second batch of imidazole (1.3 g, 19.1 mmol, 1.1 equiv.) and TBSCl (2.65 g, 17.6 mmol, 1.0 equiv.) were added and the reaction mixture was stirred at ambient temperature for 24 h, then heated to 60 °C and stirred for another 6 h. The crude mixture was allowed to cool to ambient temperature, then diluted with EtOAc (50 mL) and washed with water (100 mL x 3). The organic layer was dried over $MgSO_4$ and the solvent was removed *in vacuo*. To a solution of the crude residue in DCM (80 mL) was added dichloroacetic acid (2.4 mL) dropwise and the reaction was stirred at ambient temperature overnight. MeOH (20 mL) was added then the solvent was removed *in vacuo*. The crude residue was purified by silica gel column

chromatography (0 to 4 % MeOH in DCM) to afford **SI-10** (2.5 g, 39% over 3 steps) as a white solid.

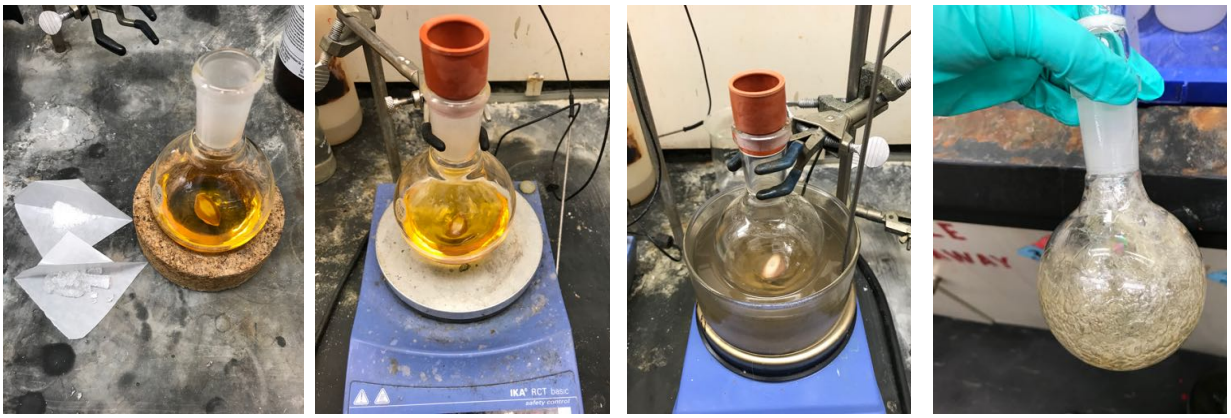
Physical State: White solid;
R_f = 0.46 (7 % MeOH in DCM);
^{1}H NMR (600 MHz, Chloroform-*d*): δ 8.33 (s, 1H), 8.07 (s, 1H), 6.28 (t, *J* = 7.3 Hz, 1H), 4.69 – 4.55 (m, 1H), 4.10 (s, 1H), 3.93 (d, *J* = 12.5 Hz, 1H), 3.76 (d, *J* = 12.5 Hz, 1H), 2.80 (s, 1H), 2.27 (d, *J* = 7.7 Hz, 1H), 0.90 (s, 9H), 0.09 (s, 6H) ppm;
^{13}C NMR (151 MHz, Chloroform-*d*): δ 158.6, 148.0, 145.8, 140.2, 125.8, 89.9, 87.1, 73.5, 62.9, 42.0, 25.9, 18.1, -4.6, -4.7 ppm;
HRMS (ESI-TOF): calcd. for $C_{16}H_{27}N_4O_4Si$ [M + H^+] 367.1802; found 367.1791.

Pictoral Guide

Synthesis of Compound SI-9

(From left to right): 4,4'-Dimethoxytrityl chloride and anhydrous pyridine, portion-wise addition of 4,4'-Dimethoxytrityl chloride to 2'-Deoxyinosine in pyridine, reaction mixture after overnight being stirred overnight.

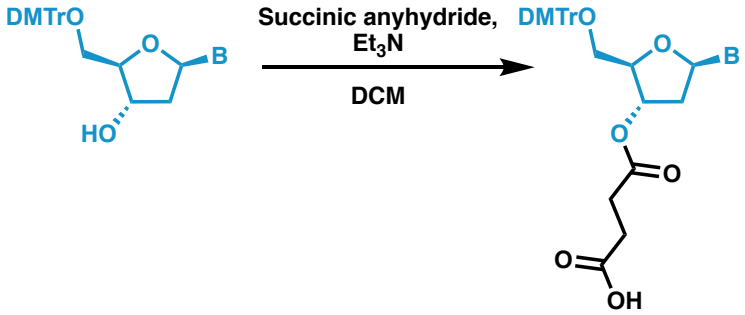
(From left to right): Crude after evaporation of pyridine, reaction workup, crude after workup and DCM evaporation.



(From left to right): Crude mixture diluted in DMF, imidazole and TBSCl, reaction mixture being stirred for 24h at rt, reaction mixture heated up to 60 °C and stirred for 6 h., crude after workup.

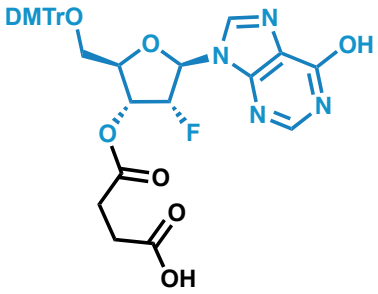
(From left to right): Crude mixture diluted in DCM, following by dropwise addition of DCA, reaction mixture after being stirred overnight, crude after evaporation DCM, dry loading of the mixture onto column, desired product after purification.

General Procedure 3 – Synthesis of Nucleoside Monomer Succinates



To a solution of the protected nucleoside (1.0 equiv.) and succinic anhydride (1.5 equiv.) in DCM was added trimethylamine (3.0 equiv.) and the reaction mixture was stirred for 3 h. The crude reaction mixture was added to triethylammonium phosphate buffer (30 mL, 0.5 M, pH 7.4) then the aqueous solution was extracted with DCM (3 x 30 mL). The combined organic layers were concentrated *in vacuo*. The crude product was used without further purification.

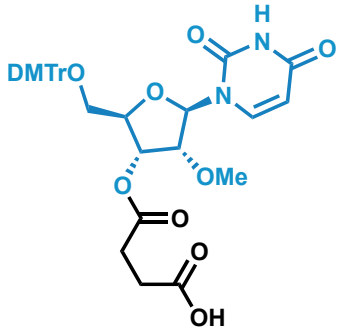
Compound SI-11



SI-11

Compound **SI-11** was prepared according to *General Procedure 3* using 9-((2*R*,3*R*,4*R*,5*R*)-5-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-3-fluoro-4-hydroxytetrahydrofuran-2-yl)-9*H*-purin-6-ol (0.42 g, 0.73 mmol, 1 equiv.) and used directly in the next reaction.

Compound SI-12

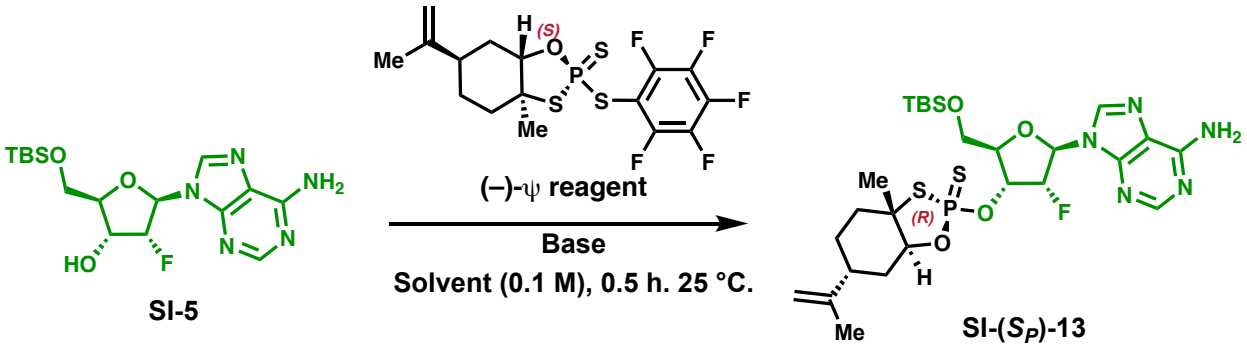


SI-12

Compound **SI-12** was prepared according to *General Procedure 3* using 1-((2*R*,3*R*,4*R*,5*R*)-5-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-4-hydroxy-3-methoxytetrahydrofuran-2-yl)pyrimidine-2,4(1*H*,3*H*)-dione (0.497 g, 0.887 mmol, 1 equiv.) and used directly in the next reaction.

Optimization of Loading Reaction

General reaction scheme



HPLC Assay

Standard Method for Assay Development

1. Prepare 0.01 M standard solutions of product (63 mg in 10 mL) and caffeine (19.2 mg in 10 mL) in DMF.
2. Add 200 µL of the caffeine standard solution, 200 µL standard solution of desired analyte, 600 µL DMF, and 500 µL water to an HPLC vial.
3. Repeat steps 1–2 for a total of 3 samples.

This enables the calculation of the conversion factor based on a 1:1 caffeine:analyte:

$$Conversion\ factor = \frac{Peak\ Area\ Analyte}{Peak\ Area\ Caffeine}$$

4. Calculate the conversion factor for each sample and calculate the average conversion factor for the 3 samples.

For standard sampling of reaction mixtures, the ratio of Caffeine:Analyte is 1:5. Therefore, when calculating the % conversion

$$\frac{Peak\ Area\ Analyte}{Peak\ Area\ Caffeine\ \times 5} = X$$

$$\frac{X}{Average\ Conversion\ Factor} = \%\ Conversion$$

Standard Method for Sampling (based on 0.05 mmol scale)

1. Prepare a 0.02 M standard Caffeine solution (192 mg caffeine in 50 mL DMF)
2. Add 0.5 mL of the caffeine standard to the reaction mixture and agitate + 2 mL DMF

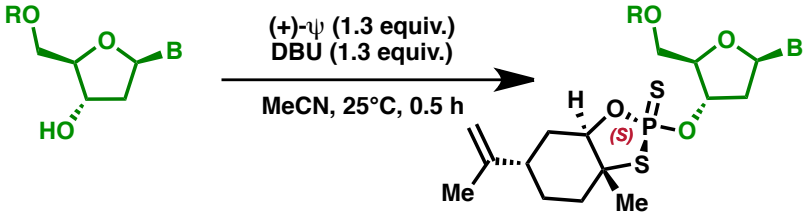
Following *General Procedure 4* using Compound **SI-5** (19.2 mg, 0.05 mmol, 1 equiv.), (–)-PSI reagent (see table for mass and equiv.), and base (see table for mass and equiv.) in solvent (0.5 mL, 0.1 M).

Optimization table (Table S4)

#	PSI Eq.	PSI mg	Solvent	Base	Base Eq.	Base mg/µL	HPLC Yield
1	1.5	33.5	MeCN	DBU	1.5	11.2 µL	71
2	1.5	33.5	DMF	DBU	1.5	11.2 µL	63
3	1.5	33.5	THF	DBU	1.5	11.2 µL	79
4	1.5	33.5	MeCN	Et_3N	1.5	10.5 µL	3
5	1.5	33.5	MeCN	DABCO	1.5	8.2 µL	35
6	1.5	33.5	MeCN	TMG	1.5	9.4 µL	58
7	1.5	33.5	MeCN	KO^tBu	1.5	8.4 mg	18
8	1.5	33.5	MeCN	Barton s	1.5	12.8 mg	62
9	1.5	33.5	MeCN	Hunigs	1.5	13.1 µL	39
10	1.5	33.5	MeCN	Phosphazene	1.5	22.9 µL	47
11	1.5	33.5	MeCN	DBU	2	14.9 µL	66
12	1.5	33.5	MeCN	DBU	1.3	9.7 µL	78
13	1.5	33.5	MeCN	DBU	1	7.5 µL	72
14	1.3	29.0	MeCN	DBU	1.3	9.7 µL	76
15	1	22.3	MeCN	DBU	1.3	9.7 µL	56

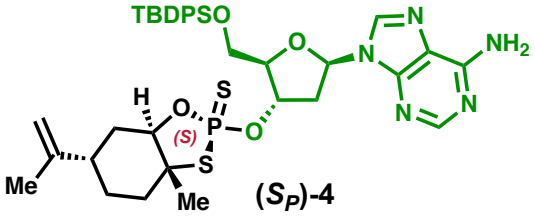
Table S4: Optimization table with HPLC yields of **SI-(*S*$_P$)-13**.

General Procedure 4 – Synthesis of Loaded Nucleosides



The loaded nucleosides were prepared as follows unless otherwise stated. Nucleoside (1.0 equiv.) and **(+) or (–)-1** (1.3 equiv.) were dissolved in anhydrous acetonitrile (0.1 M) in a flame-dried round-bottom flask. DBU (1.3 equiv.) was added dropwise to the reaction mixture while stirring. Reaction progress was monitored by ^{31}P NMR. After 30 minutes, the crude reaction mixture was filtered through a short pad of silica gel (approximately 1 inch) then the silica gel was washed with EtOAc (4 x 5 mL). The filtrate was washed with saturated aqueous $NaHCO_3$ (30 mL), water (30 mL), saturated aqueous KH_2PO_4 (30 mL), dried over $MgSO_4$ and the solvent was removed *in vacuo*. The crude product was purified by silica gel column chromatography unless otherwise stated.

Compound (S_P)-4

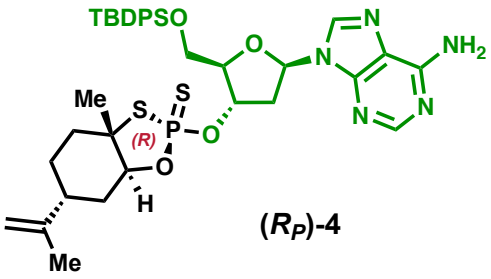


To a 250 mL flask were added nucleoside **SI-1** (4.14 g, 8.46 mmol, 1 equiv.) and the (+)-ψ reagent (4.93 g, 11.0 mmol, 1.3 equiv.) in THF (42 mL). Then, DBU (1.60 mL, 10.6 mmol, 1.3 equiv.) was added dropwise at ambient temperature. After 20 minutes, the reaction was quenched with AcOH (1.5 mL, 26 mmol, 3.1 equiv.) and stirred vigorously under air for 5 h. The crude mixture was filtered and concentrated to a paste that was purified by flash column chromatography (0% to 100% EtOAc in DCM) to afford the desired product **(S_P)-4** as a white solid (6.01 g, 97% yield).

Physical State: White solid;
^{1}H NMR (400 MHz, Chloroform-*d*): δ 8.23 (s, 1H), 8.07 (s, 1H), 7.76 - 7.54 (m, 4H), 7.48 - 7.31 (m, 6H), 6.87 (br s, 2H), 6.50 (t, *J*=7.1 Hz, 1H), 5.70 - 5.59 (m, 1H), 5.04 (s, 1H), 4.89 (s, 1H), 4.48 (dt, *J*=12.6, 3.2 Hz, 1H), 4.41 - 4.33 (m, 1H), 3.98 - 3.89 (m, 2H), 2.88 - 2.73 (m, 2H), 2.59 (br s, 1H), 2.27 (br d, *J*=13.1 Hz, 1H), 2.17 - 2.07 (m, 3H), 1.97 - 1.80 (m, 2H), 1.78 (s, 3H), 1.71 (s, 3H), 1.07 (s, 9H);
^{13}C NMR (101 MHz, Chloroform-*d*): δ 155.4, 152.0, 149.3, 144.8, 138.5, 135.6, 135.4, 132.6, 132.2, 128.98, 128.97, 127.9, 119.1, 112.0, 86.4 (d, *J* = 3.4 Hz), 86.0, 84.3, 79.6 (*J* = 8.0 Hz), 65.7, 63.6, 39.6 (d, *J* = 7.3 Hz), 38.8, 33.8, 33.7, 27.8, 27.6, 26.9, 23.4, 22.7, 21.7, 19.2;
^{31}P NMR (162 MHz, Chloroform-*d*): δ 101.0;

HRMS (ESI-TOF, m/z): HRMS (ESI) Calcd for $[C_{36}H_{46}N_5O_4PS_2Si+H]^+$ 736.2571; Found 736.2584 (1.7 ppm error).
R_f = 0.45 (25% EtOAc in DCM); UV, $KMnO_4$.

Compound (R_P)-4

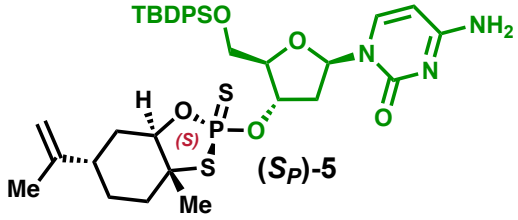


(R_P)-4

To a 250 mL flask were added nucleoside **SI-1** (3.89 g, 7.95 mmol, 1 equiv.) and the (–)-ψ reagent (4.61 g, 10.3 mmol, 1.3 equiv.) in THF (40 mL). DBU (1.50 mL, 9.9 mmol, 1.3 equiv.) was then added dropwise at ambient temperature. After 20 minutes, the reaction was quenched with AcOH (1.4 mL, 24 mmol, 3.0 equiv.) and stirred vigorously under air for 5 h. The crude was filtered and concentrated to a paste that was purified by flash column chromatography (0% to 100% EtOAc in DCM) to afford the desired product **(R_P)-4** as a white solid (5.59 g, 96% yield).

Physical State: White solid;
M.P. 181.6 °C
^{1}H NMR (400 MHz, Chloroform-*d*): δ δ 8.31 (s, 1H), 8.05 (s, 1H), 7.72 - 7.59 (m, 4H), 7.45 - 7.33 (m, 6H), 6.50 (dd, *J*=8.6, 5.6 Hz, 1H), 6.07 (s, 2H), 5.64 (br dd, *J*=11.4, 5.3 Hz, 1H), 5.06 (s, 1H), 4.92 (s, 1H), 4.49 (dt, *J*=12.5, 3.1 Hz, 1H), 4.36 - 4.29 (m, 1H), 3.91 (d, *J*=3.3 Hz, 2H), 2.87 - 2.70 (m, 2H), 2.60 (br s, 1H), 2.33 (br d, *J*=13.1 Hz, 1H), 2.19 - 2.08 (m, 1H), 2.02 - 1.83 (m, 3H), 1.83 - 1.69 (m, 7H), 1.07 (s, 9H);
^{13}C NMR (101 MHz, Chloroform-*d*): δ 155.5, 152.9, 149.7, 144.7, 138.3, 135.6, 135.4, 132.6, 132.3, 129.95, 129.92, 127.9, 119.8, 112.1, 85.96 (d, *J* = 6.6 Hz), 85.95, 83.8, 79.1 (d, *J* = 7.3 Hz), 65.8, 63.6, 39.7 (d, *J* = 4.4 Hz), 38.8, 33.7, 33.6, 27.8, 27.6, 26.9, 23.3, 22.7, 21.7, 19.2;
^{31}P NMR (162 MHz, Chloroform-*d*): δ 101.1 (s, 1P);
HRMS (ESI-TOF, m/z): HRMS (ESI) Calcd for $[C_{36}H_{46}N_5O_4PS_2Si+H]^+$ 736.2571; Found 736.2581 (1.4 ppm error).
R_f = 0.35 (25% EtOAc in DCM); UV, $KMnO_4$.

Compound (S_P)-5



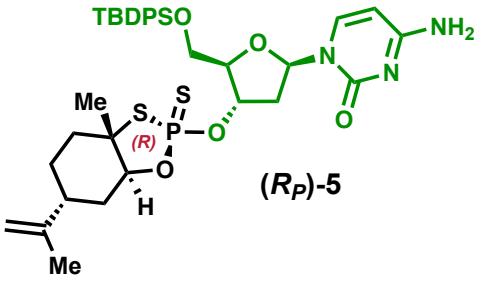
(S_P)-5

To a solution of nucleoside **SI-2** (3.20 g, 6.89 mmol, 1.0 equiv) and (–)-ψ reagent (4.20 g, 9.41 mmol, 1.5 equiv) and in THF (46 mL) was added DBU (1.35 mL, 8.98 mmol, 1.35 equiv) at

0 °C. After 1 h, the mixture was diluted with EtOAc, DCM, and heptane (60 mL each). The mixture was then washed with K_2HPO_4 (10% aq., 65 mL then 35 mL). The organic layer was dried over $MgSO_4$, and concentrated. The resulting residue was purified by flash chromatography (0 to 10% MeOH in DCM) to afford the desired product **(S_P)-5** as a white solid (4.3 g, 87% yield).

Physical State: White solid;
^{1}H NMR (400 MHz, Chloroform-*d*): δ 7.94 - 7.85 (m, 1H), 7.71 - 7.62 (m, 4H), 7.54 - 7.34 (m, 7H), 6.49 - 6.42 (m, 1H), 5.58 - 5.46 (m, 2H), 5.05(s, 1H), 4.88 (s, 1H), 4.45 (dt, J=12.4, 3.2 Hz, 1H), 4.27 (br d, J=2.0 Hz, 1H), 4.04 - 3.92 (m, 2H), 2.74 (ddd, J=14.1, 5.6, 1.8 Hz, 1H), 2.58 (br s, 1H), 2.31 - 2.09 (m, 3H), 2.04 - 1.74 (m, 8H), 1.70 (s, 3H), 1.08 (s, 9H);
^{13}C NMR (101 MHz, Chloroform-*d*): δ 144.6, 141.4, 135.7, 135.4, 132.8, 131.9, 130.2, 130.0, 128.1, 128.0, 112.2, 94.3, 86.1, 79.1, 65.6, 63.6, 40.3, 38.8, 33.8, 27.8, 27.0, 23.4, 22.6, 21.7, 19.3;
^{31}P NMR (162 MHz, Chloroform-*d*): δ 101.4;
HRMS (ESI-TOF, m/z): HRMS (ESI) Calcd for $[C_{35}H_{46}N_3O_5PS_2Si + H]^+$ 712.2459; Found 712.2470 (1.6 ppm error).
R_f = 0.52 (10% MeOH in DCM); UV, $KMnO_4$.

Compound (R_P)-5



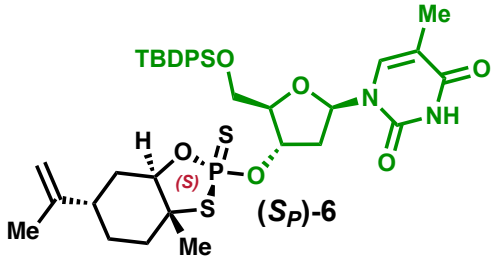
(R_P)-5

To a solution of nucleoside **SI-2** (3.5 g, 7.5 mmol, 1.0 equiv) and (+)-ψ reagent (4.9 g, 11 mmol, 1.5 equiv) in THF (60 mL, 20 v) was added DBU (1.52 mL, 10.1 mmol. 1.35 equiv) at 0 °C. After 1 h, the mixture was diluted with EtOAc, DCM, and heptane (each 60 mL). The mixture was then washed with K_2HPO_4 (10% aq., 65 mL then 35 mL. The organic layer was dried over $MgSO_4$, and concentrated. The resulting residue was purified by flash chromatography (0 to 10% MeOH in DCM) to afford the desired product **(R_P)-5** as a white solid (4.2 g, 82% yield).

Physical State: White solid;
^{1}H NMR (400 MHz, Chloroform-*d*): δ 7.93 (d, J=7.3 Hz, 1H), 7.67 (ddd, J=7.8, 3.9, 1.5 Hz, 4H), 7.53 - 7.34 (m, 7H), 6.45 (dd, J=7.3, 5.8 Hz, 1H), 5.60 - 5.33 (m, 3H), 5.08 (s, 1H), 4.92 (s, 1H), 4.47 (dt, J=12.6, 3.3 Hz, 1H), 4.23 (br d, J=2.5 Hz, 1H), 4.02 - 3.90 (m, 2H), 2.73 (ddd, J=14.0, 5.7, 2.5 Hz, 1H), 2.63 - 2.57 (m, 1H), 2.35 (br d, J=11.6 Hz, 1H), 2.28 - 2.10 (m, 2H), 2.10 - 1.95 (m, 1H), 1.94 - 1.86 (m, 2H), 1.85 - 1.77 (m, 4H), 1.71 (s, 3H), 1.08 (s, 9H);
^{13}C NMR (101 MHz, Chloroform-*d*): δ 165.1, 155.4, 144.6, 141.1, 135.7, 135.4, 132.8, 132.0, 130.1, 130.0, 128.0 (d, J=10.9 Hz, 1C), 112.2, 94.2, 85.7, 85.7 (d, J=6.4 Hz, 1C), 78.3 (d, J=7.3 Hz, 1C), 65.8, 63.4, 40.4 (d, J=4.5 Hz, 1C), 38.8, 33.7 (d, J=9.1 Hz, 1C), 27.7 (d, J=15.4 Hz, 1C), 27.0, 23.4, 22.7, 21.8, 19.2;
^{31}P NMR (162 MHz, Chloroform-*d*): δ 101.5;
HRMS (ESI-TOF, m/z): HRMS (ESI) Calcd for $[C_{35}H_{46}N_3O_5PS_2Si + H]^+$ 712.2459; Found 712.2473 (2.0 ppm error).

$\mathbf{R}_f$ = 0.55 (10% MeOH in DCM); UV, $KMnO_4$.

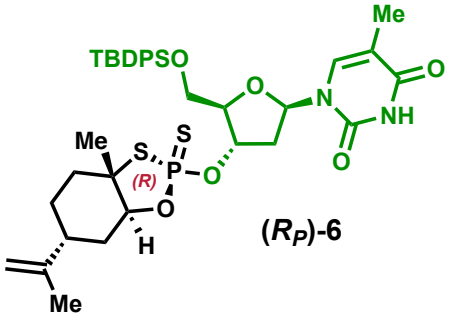
Compound (S_P)-6



A suspension of nucleoside **SI-4** (4.00 g, 8.32 mmol, 1.00 equiv.) and the (+)-ψ reagent (4.83 g, 10.8 mmol, 1.3 equiv.) in MeCN (83 mL) was cooled to an internal temperature of 0 °C. DBU (1.63 mL, 10.8 mmol, 1.3 equiv.) was added in one portion and stirred at 0 °C for 30 min. The resulting mixture was passed through a plug of silica gel (ca. 1") and washed with ethyl acetate (82 mL). The organic layer was washed with water (42 mL) then NaH_2HPO_4 (10 wt% aq., 42 mL). The organic layer was dried over $MgSO_4$, filtered and concentrated *in vacuo* to afford a yellow gel that was purified by flash column chromatography (10% to 90% EtOAc in hexanes) to afford a foam which was broken down into a powder by stirring in hexane (3 x 50 mL). This process was repeated three times total. The product **(S_P)-6** was isolated as a white powder which was dried at 50 °C and 20 Torr until constant weight was reached (5.76 g, 95 wt%, remainder hexanes and EtOAc, 90% corrected yield).

Physical State: White solid;
^{1}H NMR (400 MHz, Chloroform-*d*): δ 8.19 (br s, 1H), 7.64-7.75 (m, 4H), 7.38-7.55 (m, 7H), 6.47 (dd, J = 9.5, 5.2 Hz, 1H), 5.60 (dd, J = 11.1, 6.1 Hz, 1H), 5.07 (s, 1H), 4.90 (s, 1H), 4.48 (dt, J = 12.4, 3.3 Hz, 1H), 4.27 (s, 1H), 3.92-4.09 (m, 2H), 2.51-2.65 (m, 2H), 2.16 (td, J = 13.3, 3.9 Hz, 1H), 1.75-2.05 (m, 4H), 1.79 (s, 3H), 1.72 (s, 3H), 1.57-1.59 (m, 4H), 1.10 (s, 9H
^{13}C NMR (101 MHz, Chloroform-*d*): δ 163.4, 150.2, 144.6, 135.6, 135.2, 134.9, 132.8, 131.7, 130.2, 130.1, 128.1, 128.0, 112.2, 111.6, 86.0, 85.9 (d, J = 2.9 Hz), 84.4, 79.6 (d, J = 7.3 Hz), 65.7, 63.8, 39.2 (d, J = 8.0 Hz), 38.8, 33.7, 33.6, 27.8, 27.6, 27.0, 23.4, 22.6, 21.7, 19.4, 11.9;
^{31}P NMR (162 MHz, Chloroform-*d*): δ 101.49;
HRMS (ESI-TOF, m/z): Calcd for $[C_{36}H_{47}N_2O_6PS_2Si+H]^+$ 727.2455; Found 727.2478 (3.1 ppm error).
$\mathbf{R}_f$ = 0.41 (40 % EtOAc in hexane); UV, $KMnO_4$.

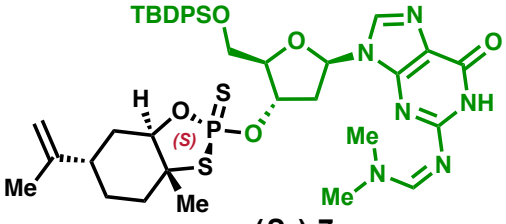
Compound (R_P)-6



A suspension of nucleoside **SI-4** (3.00 g, 6.24 mmol, 1.0 equiv.) and the (-)-ψ reagent (3.62 g, 8.11 mmol, 1.3 equiv.) in MeCN (62 mL) was cooled to 0 °C. DBU (1.22 mL, 8.11 mmol, 1.3 equiv.) was added in one portion, stirred at 0 °C for 30 min then the mixture, then passed through a plug of silica gel (ca. 1") and washed with ethyl acetate (62 mL). The solution was washed with water (31 mL) then with K_2HPO_4 (10 wt% aq., 31 mL). The organic layer was dried over $MgSO_4$, filtered and concentrated *in vacuo* to afford a yellow gel that was purified by flash column chromatography (10% to 90% EtOAc in hexanes) to afford a foam which was broken down into a powder by concentration from heptane (3 x 50 mL). The product **(R_P)-6** was isolated as a white powder which was dried at 50 °C and 20 Torr until constant weight was reached (4.46 g, 91.5 wt%, remainder heptane, 90% corrected yield).

Physical State: White solid;
^{1}H NMR (400 MHz, Chloroform-*d*): δ 8.22 (br s, 1H), 7.64-7.74 (m, 4H), 7.51 (d, J = 1.3 Hz, 1H), 7.38-7.50 (m, 6H), 6.45 (dd, J = 9.3, 5.1 Hz, 1H), 5.59 (dd, J = 11.4, 5.8 Hz, 1H), 5.09 (s, 1H), 4.93 (s, 1H), 4.50 (dt, J = 12.7, 3.3 Hz, 1H), 4.24 (d, J = 1.5 Hz, 1H), 3.97 (d, J = 2.0 Hz, 2H), 2.57-2.65 (br m, 1H), 2.57 (dd, J = 14.0, 5.4 Hz, 1H), 2.27-2.38 (m, 2H), 2.14 (td, J = 13.3, 3.9 Hz, 1H), 1.86-2.02 (m, 3H), 1.82 (s, 3H), 1.72-1.82 (m, 1H), 1.72 (s, 3H), 1.59 (d, J = 1.0 Hz, 3H), 1.10 (s, 9H);
^{13}C NMR (101 MHz, Chloroform-*d*): δ 163.6, 150.2, 144.6, 135.6, 135.2, 134.9, 132.8, 131.7, 130.2, 130.0, 128.1, 128.0, 112.2, 111.5, 86.0, 85.8 (d, J = 7.1 Hz), 84.2, 79.1 (d, J = 8.1 Hz), 65.8, 63.9, 39.5 (d, J = 4.0 Hz), 38.8, 33.7, 33.6, 27.8, 27.6, 27.0, 23.3, 22.6, 21.7, 19.3, 11.9;
^{31}P NMR (162 MHz, Chloroform-*d*): δ 101.67;
HRMS (ESI-TOF, m/z): HRMS (ESI) Calcd for $[C_{36}H_{47}N_2O_6PS_2Si+H]^+$ 727.2455; Found 727.2474 (2.6 ppm error).
R_f = 0.41 (40 % EtOAc in hexane); UV, $KMnO_4$.

Compound (S_P)-7



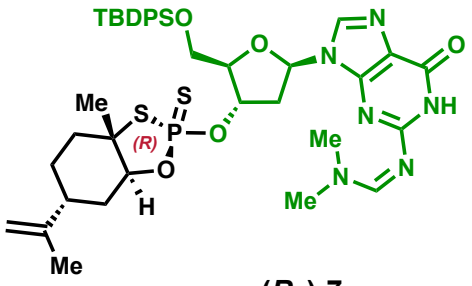
(S_P)-7

To a 100 mL flask were added **SI-3** (1.50 g, 2.60 mmol, 1.0 equiv.) and (–)-ψ (1.67 g, 3.74 mmol, 1.4 equiv.) in MeCN (27 mL). The mixture was cooled to 0 °C and DBU (0.52 mL, 3.48 mmol, 1.3 equiv.) was added dropwise; the reaction was left to stir at ambient temperature. After 1 h UPLC analysis showed complete consumption of **SI-3**. The reaction mixture was diluted with EtOAc (27 mL) then washed with water (27 mL) and Na_2HPO_4 (10 wt%, 27 mL). The organic phase was dried over Na_2SO_4, filtered and the solvent was removed *in vacuo*. The crude residue was purified by silica gel column chromatography (100% EtOAc to 100% THF) to afford the product **(S_P)-7** as a light yellow solid (1.96 g, 91%). Note: The isolated product contains BHT (from stabilized THF).

Physical State: White solid;

^{1}H NMR (400 MHz, Chloroform-*d*): δ 9.90 (br s, 1H), 8.52 (s, 1H), 7.79 (s, 1H), 7.69 - 7.60 (m, 4H), 7.44 - 7.28 (m, 6H), 6.32 (dd, J=7.7, 6.2 Hz, 1H), 5.64 (ddt, J=11.5, 6.0, 2.7 Hz, 1H), 4.99 (s, 1H), 4.87 (s, 1H), 4.47 (dt, J=12.7, 3.1 Hz, 1H), 4.28 (q, J=3.4 Hz, 1H), 3.92 - 3.82 (m, 2H), 3.12 (s, 3H), 3.06 (s, 3H), 2.90 - 2.55 (m, 4H), 2.27 - 2.22 (m, 1H), 2.18 - 2.04 (m, 1H), 2.00 - 1.80 (m, 3H), 1.76 (s, 3H), 1.73 (s, 3H), 1.04 (s, 9H);
^{13}C NMR (101 MHz, Chloroform-*d*): δ 158.4, 157.8, 156.8, 150.0, 145.0, 136.4, 135.5, 135.4, 132.5, 132.3, 129.9, 127.8, 127.8, 120.5, 111.8, 86.0, 85.8, 85.7, 83.4, 79.0, 78.9, 65.8, 63.5, 41.3, 39.0, 39.0, 38.7, 35.2, 33.7, 33.6, 27.7, 27.5, 26.8, 23.3, 22.6, 21.7, 19.1;
^{31}P NMR (162 MHz, Chloroform-*d*): δ 100.5;
HRMS (ESI-TOF, m/z): HRMS (ESI) Calcd for $[C_{39}H_{51}N_6O_5PS_2Si+H]^+$ 807.2942; Found 807.2957 (1.8 ppm error).
R_f = 0.68 (100% THF); UV, $KMnO_4$.

Compound (R_P)-7

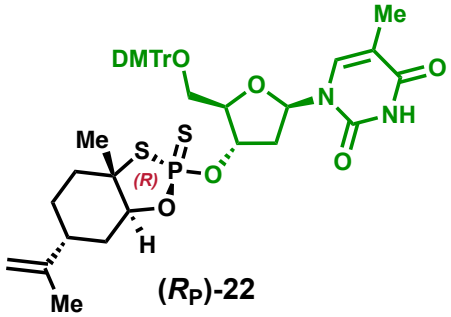


(R_P)-7

To a 100 mL flask were added **SI-3** (1.10 g, 1.96 mmol, 1.0 equiv.) and the (-)-ψ reagent (1.14 g, 2.55 mmol, 1.4 equiv.) in MeCN (20 mL). The mixture was cooled to 0 °C and DBU (0.38 mL, 2.55 mmol, 1.3 equiv.) was added dropwise and the reaction was left to stir at ambient temperature. After 1 h UPLC analysis showed complete consumption of **SI-3**. The reaction mixture was diluted with EtOAc (20 mL) then washed with water (20 mL) and Na_2HPO_4 (10 wt%, 20 mL). The organic phase was dried over Na_2SO_4, filtered, and the solvent was removed *in vacuo*. The crude residue was purified by silica gel column chromatography (0 to 100% THF in EtOAc) to afford the product **(R_P)-7** as a white solid (1.20 g, 76%). Note: The isolated product contains BHT (from stabilized THF).

Physical State: White solid;
^{1}H NMR (400 MHz, Chloroform-*d*): δ 9.61 (br s, 1H), 8.57 (s, 1H), 7.77 (s, 1H), 7.69 - 7.60 (m, 4H), 7.44 - 7.32 (m, 6H), 6.32 (dd, J=7.7, 6.2 Hz, 1H), 5.64 (ddt, J=11.5, 6.0, 2.7 Hz, 1H), 5.00 (s, 1H), 4.88 (s, 1H), 4.48 (dt, J=12.7, 3.1 Hz, 1H), 4.27 (q, J=3.4 Hz, 1H), 3.92 - 3.82 (m, 2H), 3.14 (s, 3H), 3.10 (s, 3H), 2.93 - 2.81 (m, 1H), 2.71 - 2.58 (m, 2H), 2.37 - 2.27 (m, 1H), 2.18 - 2.04 (m, 1H), 2.00 - 1.80 (m, 4H), 1.77 (s, 3H), 1.73 (s, 3H), 1.05 (s, 9H);
^{13}C NMR (101 MHz, Chloroform-*d*): δ 158.0, 157.8, 156.8, 150.0, 145.0, 136.2, 135.6, 135.4, 132.6, 132.4, 129.9, 127.8, 127.8, 120.8, 111.8, 85.9, 85.6, 85.5, 83.2, 78.5, 78.5, 65.8, 63.5, 41.3, 39.0, 39.0, 38.8, 35.2, 33.8, 33.7, 27.7, 27.6, 26.9, 23.3, 22.7, 21.7, 19.1;
^{31}P NMR (162 MHz, Chloroform-*d*): δ 100.9;
HRMS (ESI-TOF, m/z): HRMS (ESI) Calcd for $[C_{39}H_{51}N_6O_5PS_2Si+H]^+$ 807.2942; Found 807.2957 (1.8 ppm error).
R_f = 0.68 (100% THF); UV, $KMnO_4$.

Compound (R_P)-22

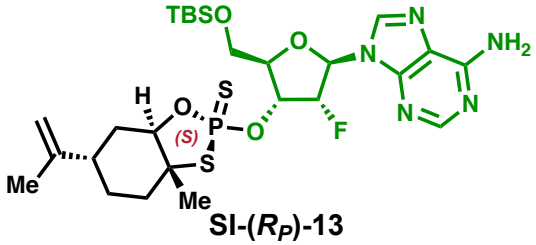


(R_P)-22

Compound **(R_P)-22** was prepared according to *General Procedure 4* using 5′-*O*-(4,4′-dimethoxytrityl)-2′-deoxythymidine (544 mg, 1.00 mmol). Purification by silica gel column chromatography (30 to 50% EtOAc in hexanes with 1% Et_3N) afforded compound **(R_P)-22** (459 mg, 58%).

Physical State: White solid;
^{1}H NMR (600 MHz, Acetone-d_6): δ 10.04 (s, 1H), 7.62 (d, *J* = 1.5 Hz, 1H), 7.54 – 7.49 (m, 2H), 7.42 – 7.31 (m, 6H), 7.30 – 7.24 (m, 2H), 6.96 – 6.90 (m, 4H), 6.38 (dd, *J* = 8.2, 6.1 Hz, 1H), 5.58 (ddt, *J* = 10.8, 5.5, 2.4 Hz, 1H), 5.02 (q, *J* = 1.5 Hz, 1H), 4.97 – 4.93 (m, 1H), 4.53 (dt, *J* = 12.8, 3.4 Hz, 1H), 4.26 (q, *J* = 3.0 Hz, 1H), 3.81 (s, 6H), 3.50 (dd, *J* = 10.6, 3.4 Hz, 1H), 3.41 (dd, *J* = 10.6, 3.2 Hz, 1H), 2.67 (s, 1H), 2.70 – 2.57 (m, 2H), 2.35 (ddd, *J* = 13.2, 3.6, 1.8 Hz, 1H), 2.12 – 2.04 (m, 1H), 2.03 – 1.94 (m, 3H), 1.89 (ddt, *J* = 13.9, 10.5, 5.6 Hz, 1H), 1.81 (s, 3H), 1.71 (s, 3H), 1.49 (d, *J* = 1.2 Hz, 3H).
^{13}C NMR (151 MHz, Acetone-d_6): δ 164.11, 159.79, 159.77, 151.20, 146.30, 145.70, 136.45, 136.25, 136.01, 131.00, 130.99, 128.96, 128.79, 127.80, 114.08, 112.18, 111.32, 87.77, 86.78, 85.10, 85.05, 85.04, 80.01, 79.96, 66.91, 64.22, 55.55, 39.77, 39.48, 39.46, 34.47, 34.41, 28.26, 28.15, 23.93, 22.79, 22.06, 12.15.
^{31}P NMR (162 MHz, Acetone-d_6): δ 101.75.
HRMS (ESI-TOF, m/z): Calcd for $C_{41}H_{47}N_2O_8PS_2$ $[M-DMTr+H]^+$489.1277.; found 489.1278.
R_f = 0.43 (5% Acetone in DCM); UV, $KMnO_4$.

Compound SI-(R_P)-13

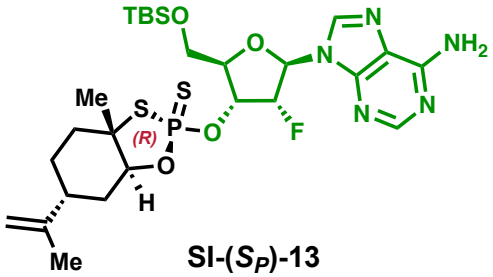


SI-(R_P)-13

Compound **SI-(R_P)-13** prepared according to *General Procedure 4* using 5'-*O*-(*tert*-butyldimethylsilyl)-2'-deoxy-2'-fluoroadenosine **SI-5** (1.37 g, 3.58 mmol). Crystallization of the crude reaction mixture from acetonitrile afforded compound **SI-(R_P)-13** (1.85 g, 82%).

Physical State: White solid;
^{1}H NMR (600 MHz, Acetone-d_6): δ 8.22 (d, J = 7.6 Hz, 2H), 6.71 (s, 2H), 6.35 (dd, J = 17.8, 2.1 Hz, 1H), 5.89 – 5.74 (m, 2H), 5.02 (q, J = 1.5 Hz, 1H), 4.97 – 4.93 (m, 1H), 4.59 (dt, J = 12.8, 3.5 Hz, 1H), 4.33 (dt, J = 6.2, 3.1 Hz, 1H), 4.06 (dd, J = 11.8, 2.8 Hz, 1H), 3.90 (dd, J = 11.8, 3.4 Hz, 1H), 2.86 (s, 1H), 2.65 (d, J = 5.8 Hz, 1H), 2.30 (dtd, J = 13.4, 3.3, 1.6 Hz, 1H), 2.12 (td, J = 13.6, 4.4 Hz, 1H), 2.05 – 1.83 (m, 4H), 1.81 – 1.78 (m, 3H), 1.70 (s, 3H), 0.89 (s, 9H), 0.07 (d, J = 22.5 Hz, 6H).
^{13}C NMR (151 MHz, Acetone-d_6): δ 157.23, 153.94, 150.28, 146.20, 140.03, 120.64, 112.05, 93.35, 92.10, 87.64, 87.42, 86.66, 82.85, 82.79, 73.87, 73.83, 73.78, 73.73, 67.33, 62.14, 39.79, 34.41, 34.35, 28.23, 28.13, 26.35, 23.93, 22.67, 22.09, 18.95, -5.20, -5.30.
^{19}F NMR (376 MHz, Acetone-d_6): δ -202.95.
^{31}P NMR (162 MHz, Acetone-d_6): δ 101.65.
HRMS (ESI-TOF, m/z): Calcd for $C_{26}H_{41}FN_5O_4PS_2Si$ [M + H]$^+$ 630.2164.; found 630.2167;
R_f = 0.43 (20 % acetone in DCM); UV, $KMnO_4$.

Compound SI-(S_P)-13

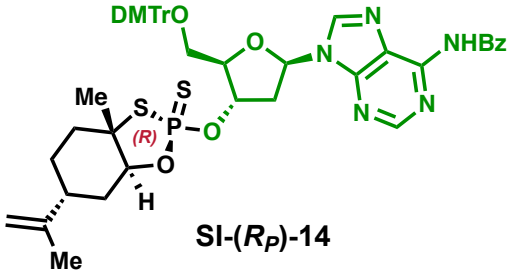


SI-(S_P)-13

Compound **SI-(S_P)-13** was prepared according to *General Procedure 4* using 5'-*O*-(*tert*-butyldimethylsilyl)-2'-deoxy-2'-fluoroadenosine **SI-5** (685 mg, 1.79 mmol). Crystallization of the crude reaction mixture from acetonitrile afforded compound **SI-(S_P)-13** (933 mg, 83%).

Physical State: White solid;
^{1}H NMR (600 MHz, Chloroform-d): δ 8.39 (s, 1H), 8.16 (s, 1H), 6.39 (dd, J = 14.3, 3.7 Hz, 1H), 5.82 (s, 2H), 5.65 – 5.49 (m, 2H), 5.06 (q, J = 1.4 Hz, 1H), 4.94 – 4.90 (m, 1H), 4.55 (ddd, J = 12.8, 3.7, 2.5 Hz, 1H), 4.44 (h, J = 2.1 Hz, 1H), 4.04 (dd, J = 11.7, 2.3 Hz, 1H), 3.90 (dd, J = 11.7, 2.7 Hz, 1H), 2.62 (s, 1H), 2.33 (ddt, J = 13.0, 3.8, 1.7 Hz, 1H), 2.18 (td, J = 13.5, 4.2 Hz, 1H), 2.04 – 1.86 (m, 3H), 1.74 (s, 3H), 0.95 (s, 7H), 0.14 (d, J = 8.8 Hz, 7H);
^{13}C NMR (151 MHz, Chloroform-d): δ 155.00, 152.90, 149.28, 144.34, 138.10, 119.43, 111.59, 91.66, 91.64, 90.35, 90.33, 85.84, 85.50, 85.29, 82.70, 82.66, 73.80, 73.75, 73.70, 73.66, 65.18, 61.27, 38.41, 33.23, 33.17, 27.33, 27.22, 25.53, 22.92, 22.23, 21.24, 17.99, -5.82, -5.86;
^{19}F NMR (376 MHz, Chloroform-d): δ -204.21;
^{31}P NMR (162 MHz, Chloroform-d): δ 103.12;
HRMS (ESI-TOF, m/z): Calcd for $C_{26}H_{41}FN_5O_4PS_2Si$ [M + H]$^+$ 630.2164.; found 630.2165;
R_f = 0.43 (20 % acetone in DCM); UV, $KMnO_4$.

Compound SI-(R_P)-14

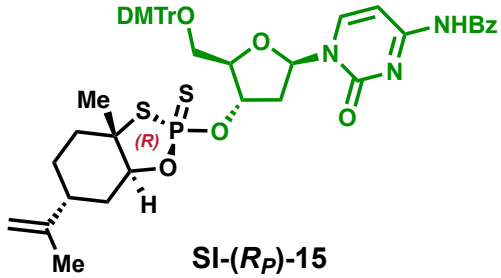


SI-(R_P)-14

Compound **SI-(R_P)-14** was prepared according to *General Procedure 4* using N^6-benzoyl-5′-*O*-(4,4′-dimethoxytrityl)-2′-deoxyadenosine (657 mg, 1.00 mmol). Purification by silica gel column chromatography (30 to 50% EtOAc in hexanes with 1% TEA) afforded compound **SI-(R_P)-14** (461 mg, 51%).

Physical State: White solid;
^{1}H NMR (600 MHz, Acetone-d_6): δ 10.00 (s, 1H), 8.58 (s, 1H), 8.42 (s, 1H), 8.14 (d, J = 7.4 Hz, 2H), 7.69 – 7.62 (m, 1H), 7.57 (t, J = 7.8 Hz, 2H), 7.54 – 7.46 (m, 2H), 7.38 – 7.26 (m, 7H), 7.25 – 7.19 (m, 1H), 6.89 – 6.82 (m, 4H), 6.58 (dd, J = 7.7, 6.1 Hz, 1H), 5.71 (ddt, J = 11.3, 5.7, 2.7 Hz, 1H), 5.05 (q, J = 1.5 Hz, 1H), 4.98 (d, J = 1.8 Hz, 1H), 4.58 (dt, J = 12.8, 3.4 Hz, 1H), 4.39 (td, J = 5.0, 2.6 Hz, 1H), 3.79 (d, J = 2.5 Hz, 6H), 3.52 – 3.35 (m, 3H), 2.87 – 2.78 (m, 1H), 2.68 (s, 1H), 2.36 (ddq, J = 13.8, 3.3, 1.6 Hz, 1H), 2.12 (td, J = 13.6, 4.5 Hz, 1H), 2.07 – 1.96 (m, 2H), 1.95 – 1.85 (m, 1H), 1.82 (s, 3H), 1.73 (s, 3H).
^{13}C NMR (151 MHz, Acetone-d_6): δ 159.75, 152.98, 152.70, 151.45, 146.60, 146.05, 143.56, 136.73, 135.21, 133.87, 133.35, 131.13, 131.07, 130.56, 129.54, 129.30, 129.11, 128.74, 127.72, 126.37, 114.06, 112.29, 87.41, 87.01, 85.63, 85.58, 85.56, 80.20, 80.15, 67.03, 64.30, 55.66, 39.95, 38.49, 38.47, 34.72, 34.66, 28.45, 28.34, 24.11, 22.99, 22.24.
^{31}P NMR (162 MHz, Acetone-d_6): δ 101.42.
HRMS (ESI-TOF, m/z): Calcd for $C_{48}H_{50}N_5O_7PS_2$ $[M + H]^+$ 904.2963.; found 904.2968;
R_f = 0.57 (5% Acetone in DCM); UV, $KMnO_4$.

Compound SI-(R_P)-15



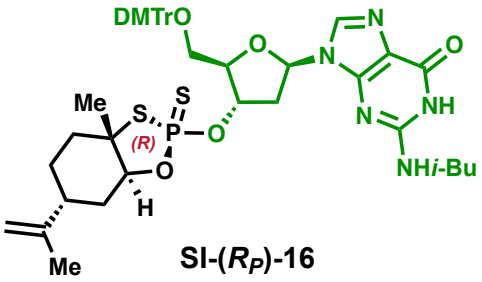
SI-(R_P)-15

Compound **SI-(R_P)-15** was prepared according to *General Procedure 4* using N^4-benzoyl-5′-*O*-(4,4′-dimethoxytrityl)-2′-deoxycytidine (634 mg, 1.00 mmol). Purification by silica gel column chromatography (30 to 50% EtOAc in hexanes with 1% Et_3N) afforded **SI-(R_P)-15** (395 mg, 45%).

Physical State: White solid;

^{1}H NMR (600 MHz, Chloroform-*d*): δ 8.68 (s, 1H), 8.20 (d, *J* = 7.5 Hz, 1H), 7.91 (d, *J* = 7.7 Hz, 2H), 7.63 (t, *J* = 7.5 Hz, 1H), 7.54 (t, *J* = 7.5 Hz, 2H), 7.41 (d, *J* = 7.2 Hz, 2H), 7.36 – 7.29 (m, 6H), 7.28 – 7.24 (m, 1H), 6.88 (dd, *J* = 8.8, 3.5 Hz, 4H), 6.37 (t, *J* = 6.5 Hz, 1H), 5.61 – 5.52 (m, 1H), 5.09 (s, 1H), 4.94 (s, 1H), 4.48 (dt, *J* = 12.7, 3.2 Hz, 1H), 4.43 – 4.39 (m, 1H), 3.82 (s, 3H), 3.81 (s, 3H), 3.55 (dd, *J* = 10.9, 3.3 Hz, 1H), 3.46 (dd, *J* = 10.9, 2.8 Hz, 1H), 2.93 (ddd, *J* = 14.4, 5.8, 2.8 Hz, 1H), 2.62 (d, *J* = 6.2 Hz, 1H), 2.46 – 2.33 (m, 2H), 2.15 (td, *J* = 13.5, 4.2 Hz, 1H), 2.03 – 1.97 (m, 1H), 1.96 – 1.88 (m, 2H), 1.85 (s, 3H), 1.82 – 1.75 (m, 1H), 1.73 (s, 3H) ppm;
^{13}C NMR (151 MHz, Chloroform-*d*): δ 162.2, 158.8, 158.8, 144.8, 144.1, 135.3, 135.1, 133.3, 130.2, 130.1, 129.2, 128.2, 127.6, 127.3, 113.5, 113.5, 112.3, 87.3, 87.2, 86.2, 85.8, 85.7, 78.6, 78.6, 66.1, 62.8, 55.4, 55.4, 40.9, 40.9, 39.0, 33.9, 33.8, 27.9, 27.8, 25.7, 23.5, 22.9, 21.9 ppm;
^{31}P NMR (162 MHz, Chloroform-*d*): *δ* 102.1 ppm;
$[\square]_D^{20}$ = –13.6 (*c* 0.50, CH_2Cl_2);
HRMS (ESI-TOF): calcd. for $C_{47}H_{51}N_3O_8PS_2$ $[M + H]^+$ 880.2855; found 880.2878;
R_f = 0.25 (30% EtOAc in hexanes); UV, $KMnO_4$.

Compound SI-(R_P)-16



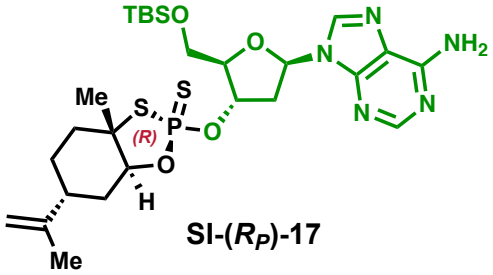
SI-(R_P)-16

Compound **SI-(R_P)-16** was prepared according to *General Procedure 4* using *N*^{6}-benzoyl-5′-*O*-(4,4′-dimethoxytrityl)-2′-deoxyadenosine (657 mg, 1.00 mmol). Purification by silica gel column chromatography (50 to 100% EtOAc in hexanes with 0.1% TEA) afforded compound **SI-(R_P)-16** (263 mg, 30%).

Physical State: White solid;
^{1}H NMR (600 MHz, Acetone-d_6): δ 10.26 (s, 1H), 7.94 (s, 1H), 7.47 – 7.42 (m, 2H), 7.34 – 7.29 (m, 4H), 7.26 (s, 1H), 7.26 – 7.17 (m, 2H), 6.87 – 6.80 (m, 4H), 6.26 (dd, *J* = 8.2, 5.8 Hz, 1H), 5.55 (ddt, *J* = 11.3, 5.5, 2.6 Hz, 1H), 5.00 (q, *J* = 1.4 Hz, 1H), 4.93 (dt, *J* = 1.9, 0.9 Hz, 1H), 4.50 (dt, *J* = 12.8, 3.4 Hz, 1H), 4.25 (td, *J* = 4.6, 2.5 Hz, 1H), 4.05 (q, *J* = 7.2 Hz, 1H), 3.77 (s, 6H), 3.44 (dd, *J* = 10.5, 5.1 Hz, 1H), 3.34 (dd, *J* = 10.4, 4.2 Hz, 1H), 3.15 (ddd, *J* = 14.1, 8.2, 5.9 Hz, 1H), 2.72 (ddd, *J* = 14.1, 5.9, 2.6 Hz, 1H), 2.66 (s, 1H), 2.36 – 2.29 (m, 1H), 2.12 – 1.94 (m, 9H), 1.88 (ddt, *J* = 15.0, 13.2, 4.9 Hz, 1H), 1.77 (dt, *J* = 1.4, 0.7 Hz, 3H), 1.71 (s, 3H), 1.25 – 1.17 (m, 8H).
^{13}C NMR (151 MHz, Acetone-d_6): δ 180.74, 180.67, 171.03, 159.80, 159.79, 155.85, 149.55, 149.24, 146.62, 146.01, 137.95, 136.72, 136.65, 131.14, 131.08, 129.12, 128.75, 127.77, 122.46, 114.06, 112.27, 87.44, 87.12, 85.73, 85.68, 84.65, 80.05, 80.00, 67.12, 64.45, 60.68, 55.65, 39.92, 38.70, 38.67, 36.80, 36.76, 34.73, 34.67, 30.50, 30.34, 30.21, 29.85, 29.70, 28.44, 28.34, 24.07, 22.94, 22.21, 20.98, 19.50, 19.36, 14.65.
^{31}P NMR (162 MHz, Acetone-d_6): δ 100.84.
HRMS (ESI) *m/z*: calculated for $C_{45}H_{52}N_5O_8PS_2$ $[M+H]^+$ 886.3068; found 886.3066.

R_f = 0.25 (60% EtOAc in hexanes + 0.1% Et_3N); UV, $KMnO_4$.

Compound SI-(R_P)-17

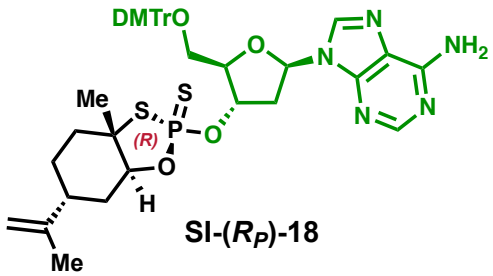


SI-(R_P)-17

Compound **SI-(R_P)-17** was prepared according to *General Procedure 4* using 5'-*O*-(*tert*-butyldimethylsilyl)-2'-deoxyadenosine (585 mg, 1.6 mmol). Purification by flash column chromatography (70% EtOAc in hexanes) afforded compound **SI-(R_P)-17** (628 mg, 64% yield).

Physical State: White solid;
^{1}H NMR (600 MHz, Acetone-d_6): δ 8.21 (d, *J* = 7.5 Hz, 2H), 6.61 (s, 1H), 6.49 – 6.41 (m, 1H), 5.54 (ddt, *J* = 10.4, 5.6, 2.2 Hz, 1H), 5.06 (q, *J* = 1.5 Hz, 1H), 5.01 – 4.98 (m, 1H), 4.57, (dt, *J* = 12.8, 3.4 Hz, 1H), 4.26 (td, *J* = 4.5, 2.0 Hz, 1H), 3.98 (dd, *J* = 11.1, 5.0 Hz, 1H), 3.89 (dd, *J* = 11.2, 4.0 Hz, 1H), 3.11 (ddd, *J* = 14.0, 8.3, 5.7 Hz, 1H), 2.74 (ddd, *J* = 14.2, 5.9, 2.3 Hz, 1H), 2.68 (s, 1H), 2.37 (ddt, *J* = 13.4, 3.3, 1.7 Hz, 1H), 2.13 (td, *J* = 13.6, 4.4 Hz, 1H), 2.05 – 1.95 (m, 5 H), 1.89 (tdd, *J* = 14.8, 6.0, 4.5 Hz, 1H), 1.82 (s, 3H), 1.71 (s, 3H), 0.95 (s, 1H), 0.93 (s, 9H), 0.17 (d, *J* = 2.7 Hz, 1H), 0.12 (s, 6H), 0.08 (d, *J* = 8.0 Hz, 1H).
^{13}C NMR (151 MHz, Acetone-d_6): δ 157.31, 153.89, 150.76, 146.59, 139.92, 112.34, 87.03, 86.70, 85.04, 80.47, 73.55, 67.02, 64.06, 39.98, 39.35, 34.64, 28.36, 26.53, 24.12, 22.99, 22.23, 19.10, –5.04.
^{31}P NMR (162 MHz, Acetone-d_6): δ 100.35.
HRMS (ESI) *m/z*: calculated for $C_{26}H_{42}N_5O_4PS_2Si$ $[M+H]^+$ 612.22*58*; found 612.2258.
R_f = 0.31 (60% EtOAc in hexanes + 0.1% Et_3N); UV, $KMnO_4$.

Compound SI-(R_P)-18



SI-(R_P)-18

Compound **SI-(R_P)-18** was prepared according to *General Procedure 4* using 5'-*O*-(*tert*-butyldimethylsilyl)-2'-deoxyadenosine (516 mg, 0.93 mmol). Purification by flash column chromatography (20% Acetone in DCM) afforded compound **SI-(R_P)-18** (454 mg, 58% yield).

Physical State: White solid;
^{1}H NMR (600 MHz, Acetone-d_6): δ 8.13 (d, *J* = 4.4 Hz, 2H), 7.50 – 7.44 (m, 2H), 7.37 – 7.31 (m, 4H), 7.27 (t, *J* = 7.7 Hz, 2H), 7.24 – 7.16 (m, 1H), 6.87 – 6.80 (m, 4H), 6.67 (s, 2H), 6.44 (dd, *J* = 7.9, 6.1 Hz, 1H), 5.65 (ddt, *J* = 11.2, 5.6, 2.6 Hz, 1H), 5.03 (q, *J* = 1.5 Hz, 1H), 4.97 –

4.94 (m, 1H), 4.54 (dt, *J* = 12.8, 3.4 Hz, 1H), 4.32 (td, *J* = 5.0, 2.5 Hz, 1H), 3.77 (d, *J* = 1.5 Hz, 6H), 3.47 (dd, *J* = 10.3, 5.2 Hz, 1H), 3.39 (dd, *J* = 10.3, 5.0 Hz, 1H), 3.32 (ddd, *J* = 14.0, 7.9, 5.9 Hz, 1H), 2.72 (ddd, *J* = 14.2, 6.1, 2.7 Hz, 1H), 2.66 (s, 1H), 2.34 (ddq, *J* = 13.4, 3.1, 1.6 Hz, 1H), 2.09 (s, 1H), 2.04 – 1.96 (m, 3H), 1.88 (dddd, *J* = 14.9, 13.5, 6.0, 4.5 Hz, 1H), 1.80 (s, 3H), 1.70 (s, 3H).

^{13}C NMR (151 MHz, Acetone-d_6): δ 158.73, 158.71, 156.23, 152.72, 149.65, 145.54, 145.06, 139.47, 135.74, 130.11, 130.06, 128.10, 127.70, 126.67, 119.99, 113.01, 111.27, 86.36, 85.94, 84.42, 84.36, 84.24, 79.34, 79.29, 65.97, 63.30, 54.61, 38.91, 37.48, 37.46, 33.68, 33.62, 29.72, 27.40, 27.30, 23.07, 21.95, 21.20.

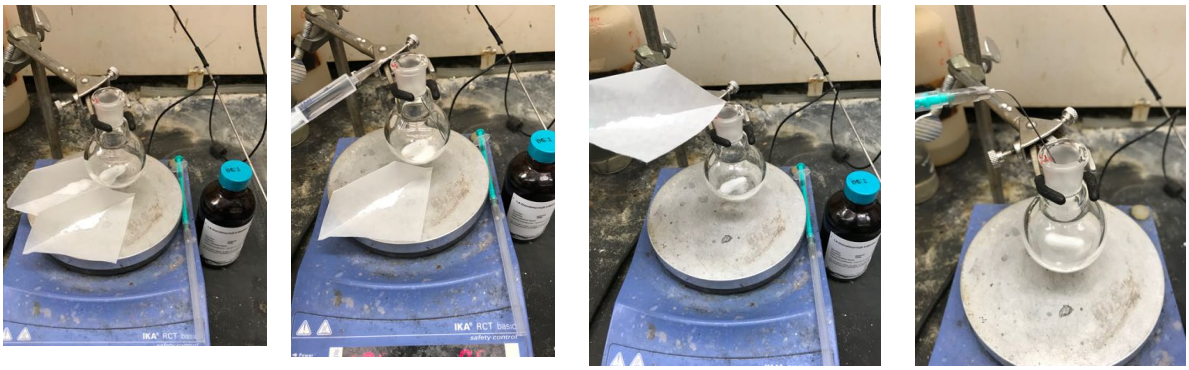
^{31}P NMR (162 MHz, Acetone-d_6): δ 100.33.

HRMS (ESI) *m/z*: calculated for $C_{41}H_{46}N_5O_6PS_2$ $[M+H]^+$ 800.2700; found 800.2701.

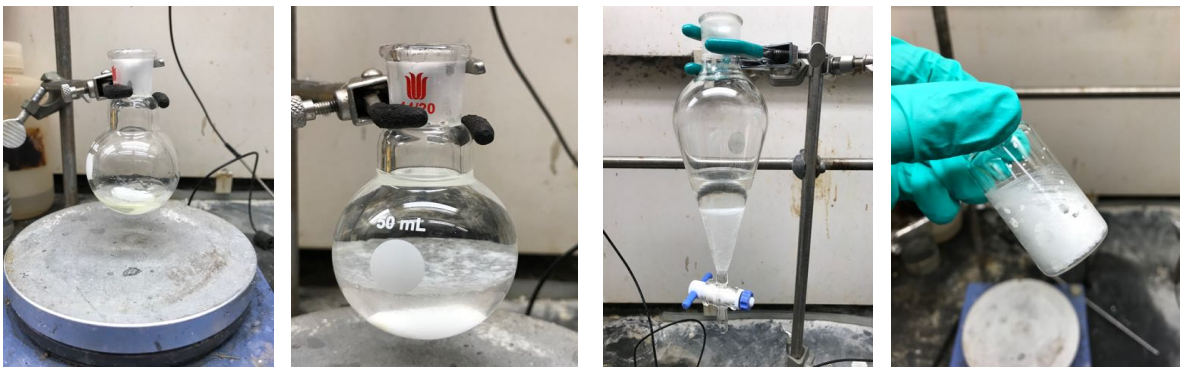
R_f = 0.29 (20% Acetone in DCM)

Pictoral Guide

General Procedure 4 (Homogeneous Reaction)

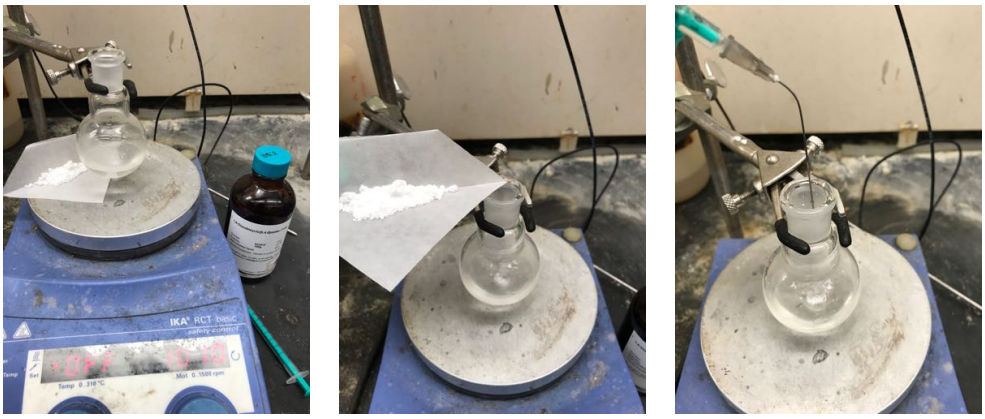


(From left to right): Nucleoside, PSI reagent, DBU, nucleoside diluted in MeCN, addition of PSI reagent, addition of DBU.



(From left to right): Reaction mixture after being stirred for 30 minutes, reaction mixture diluted with EtOAc and washed with $NaHCO_3$, reaction workup, final product after flash column purification.

General Procedure 4 (Heterogeneous Reaction)

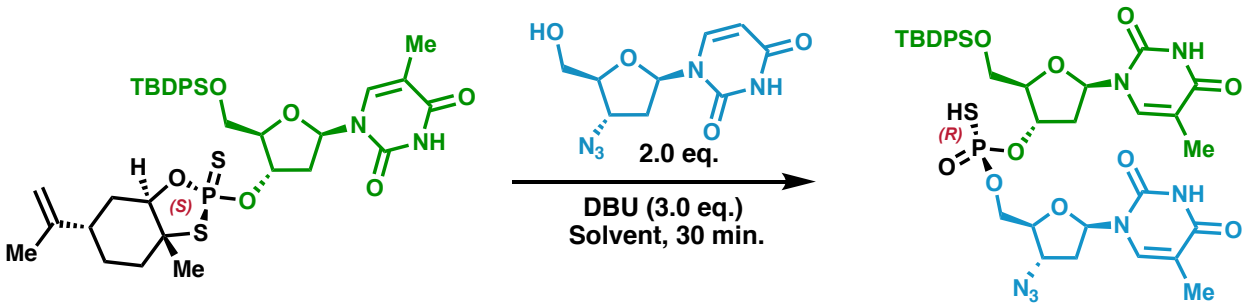


(From left to right): Nucleoside diluted in MeCN, PSI reagent, DBU, addition of PSI reagent, addition of DBU.

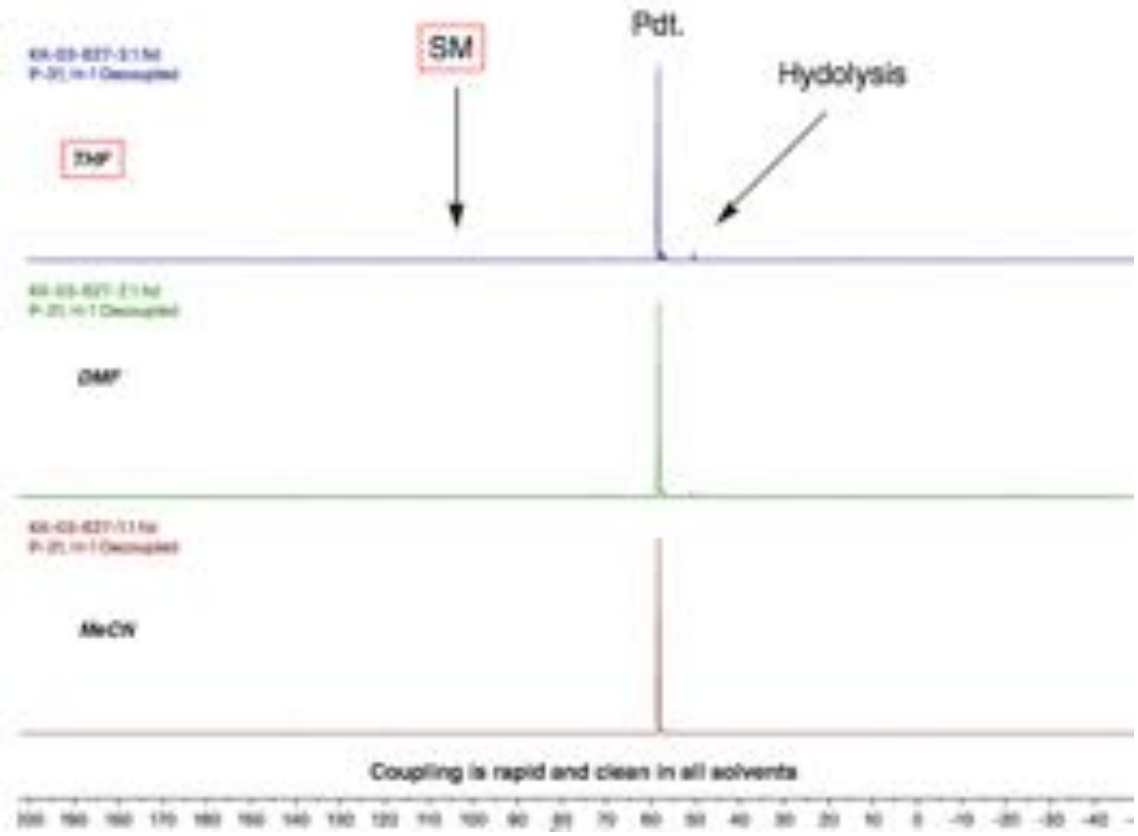
(From left to right): Reaction mixture after being stirred for 10 minutes, heterogeneous mixture before filtration, final desired product after filtration.

Optimization of Coupling Reaction

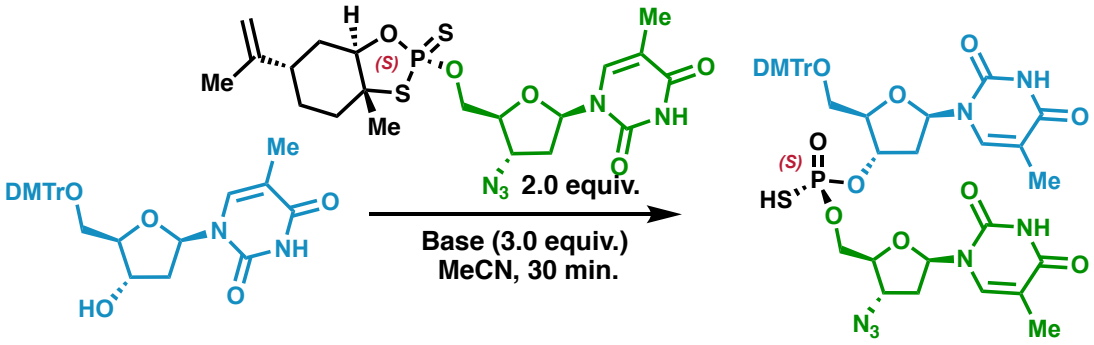
Solvent Screen



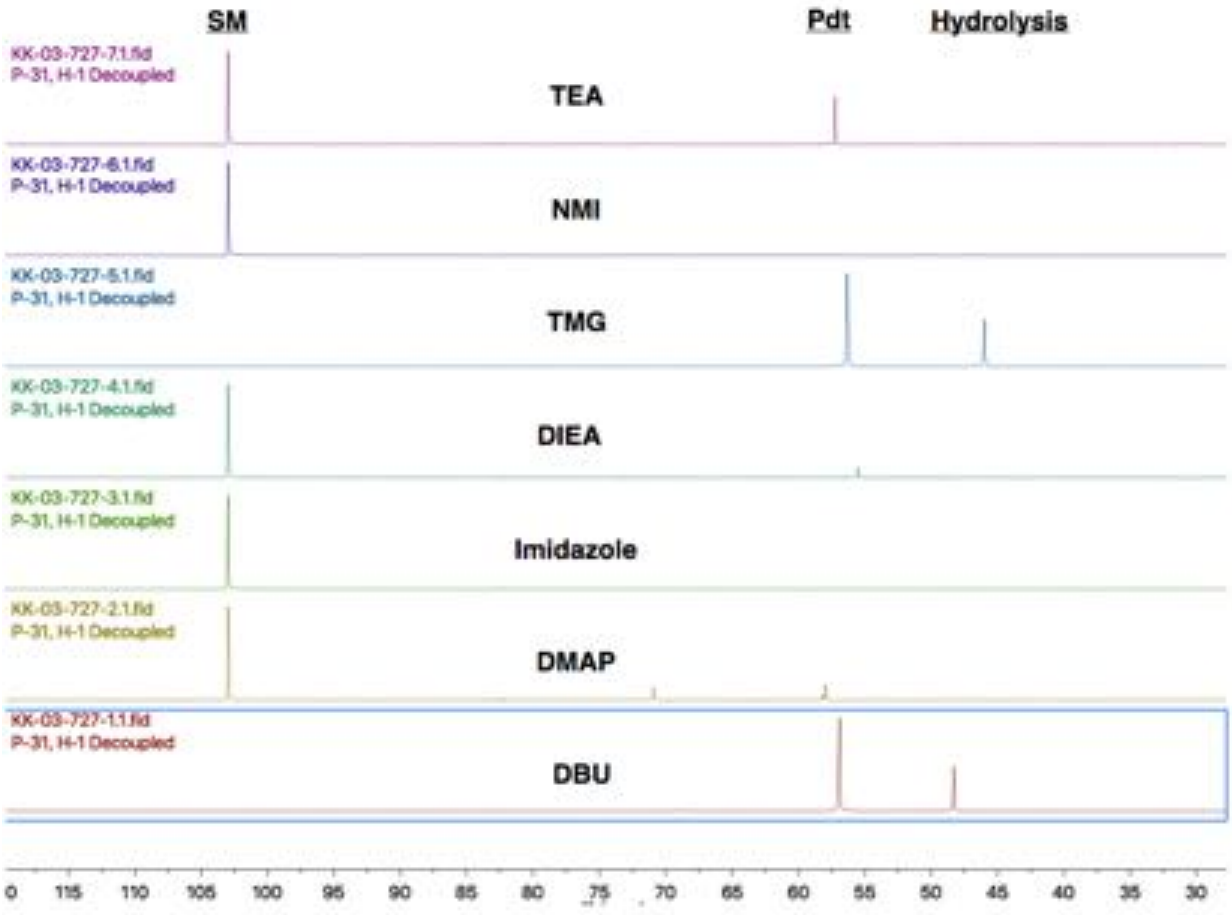
Crude ^{31}P NMR analysis



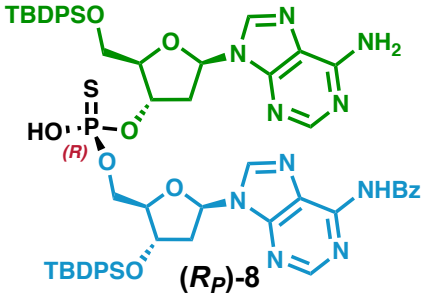
Base Screen



Crude ^{31}P NMR analysis



Compound (R_P)-8

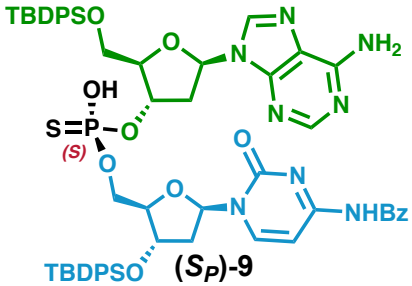


(R_P)-8

To a 50 mL flask were added compound **(S_P)-4** (535 mg, 0.73 mmol, 1.0 equiv.) and nucleoside **SI-9** (0.88 g, 1.5 mmol, 2.0 equiv.) in THF (5 mL). DBU (0.33 mL, 2.19 mmol, 3 equiv.) was then added dropwise and the reaction was left to stir at ambient temperature. After 10 minutes UPLC analysis showed complete consumption of compound **(S_P)-4**. The reaction mixture was diluted with EtOAc (10 mL), DCM (5 mL) and 20% citric acid (5 mL). The organic phase was washed with brine (5 mL), dried over Na_2SO_4, filtered and the solvent was removed *in vacuo*. The crude residue was purified by silica gel column chromatography (0 to 100% MeOH in DCM) to afford the product **(R_P)-8** as a white solid (548 mg, 65%).

Physical State: White solid;
^{1}H NMR (400 MHz, DMSO-d_6): δ 11.1 (br s, 1H), 8.84 (s, 1H), 8.71 (s, 1H), 8.21 (s, 1H), 8.08 (s, 1H), 8.05 - 8.00 (m, 2H), 7.72 - 7.47 (m, 12H), 7.45 - 7.27 (m, 11H), 6.62 (dd, *J*=8.5, 5.9 Hz, 1H), 6.27 (dd, *J*=8.5, 6.0 Hz, 1H), 5.10 (br dd, *J*=6.2, 3.4 Hz, 1H), 4.69 (br s, 1H), 4.19 (br d, *J*=16.4 Hz, 2H), 3.89 (br dd, *J*=11.1, 4.0 Hz, 2H), 3.80 - 3.66 (m, 2H), 2.82 - 2.65 (m, 2H), 2.48 - 2.23 (m, 2H), 1.06 (s, 9H), 0.93 (s, 9H);
^{13}C NMR (101 MHz, DMSO-d_6): δ 151.9, 150.1, 148.8, 142.9, 139.1, 135.1, 134.9, 134.8, 134.3, 133.3, 132.7, 132.6, 132.6, 132.4, 132.2, 129.9, 129.8, 129.6, 129.6, 128.3, 128.2, 127.7, 127.6, 127.4, 125.3, 118.9, 86.4, 85.9, 83.4, 75.1, 74.6, 64.8, 64.7, 63.9, 48.4, 40.2, 39.9, 37.3, 30.5, 26.6, 26.5, 26.4, 18.6, 18.5;
^{31}P NMR (162 MHz, DMSO-d_6): δ 53.8;
HRMS (ESI-TOF, m/z): Calcd for $[C_{59}H_{65}N_{10}O_8PSSi_2 + H]^+$ 1161.4057; Found 1161.4094 (3.3 ppm error).
R_f = 0.30 (10% MeOH in DCM); UV, $KMnO_4$.

Compound (S_P)-9



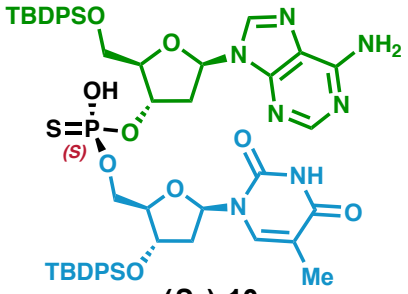
(S_P)-9

To a 50 mL flask were added compound **(R_P)-4** (1.00 g, 1.36 mmol, 1.0 equiv.) and nucleoside **SI-7** (1.55 g, 2.72 mmol, 2.0 equiv.) in THF (3 mL) and MeCN (20 mL). DBU (0.41 mL, 2.72 mmol, 2 equiv.) was then added dropwise and the reaction was left to stir at ambient temperature. After

1 hour UPLC analysis showed complete consumption of compound **(R_P)-4**. The reaction mixture was diluted with EtOAc (25 mL) and 20% citric acid (25 mL). Then the organic phase was washed with brine (25 mL), dried over Na_2SO_4, filtered and the solvent was removed *in vacuo*. The crude residue was purified by silica gel column chromatography (0 to 35% MeOH in EtOAc) to afford the product **(S_P)-9** as a white solid (1.41 g, 91%).

Physical State: White solid;
^{1}H NMR (400 MHz, DMSO-d_6): δ 11.21 (br s, 1H), 8.55 (d, J=7.3 Hz, 1H), 8.22 (s, 1H), 8.09 (s, 1H), 8.00 - 7.95 (m, 2H), 7.63 - 7.54 (m, 9H), 7.53 - 7.26 (m, 17H), 6.38 (t, J=6.5 Hz, 1H), 6.32 (t, J=6.7 Hz, 1H), 5.18 - 5.11 (m, 1H), 4.48 (br d, J=4.3 Hz, 1H), 4.23 - 4.03 (m, 2H), 3.91 - 3.78 (m, 2H), 3.73 (br dd, J=11.1, 4.8 Hz, 1H), 3.68 - 3.52 (m, 1H), 2.89 - 2.78 (m, 1H), 2.57 - 2.50 (m, 1H), 2.28 (br dd, J=12.5, 5.4 Hz, 1H), 1.94 (ddd, J=13.3, 8.3, 5.2 Hz, 1H), 1.02 (s, 9H), 0.93 (s, 9H);
^{13}C NMR (101 MHz, DMSO-d_6): δ 167.3, 163.0, 155.3, 154.4, 151.7, 149.1, 145.5, 139.1, 136.4, 135.2, 135.2, 135.1, 135.0, 134.5, 133.2, 132.8, 132.7, 132.7, 132.5, 130.0, 129.8, 129.7, 129.2, 128.4, 128.0, 128.0, 127.8, 127.7, 127.5, 119.1, 96.7, 86.8, 86.8, 86.3, 85.9, 85.8, 83.6, 75.0, 75.0, 74.5, 64.5, 64.4, 64.1, 41.2, 38.0, 26.7, 26.6, 18.7, 18.6;
^{31}P NMR (162 MHz, DMSO-d_6): δ 54.1;
HRMS (ESI-TOF, m/z): Calcd for $[C_{58}H_{65}N_8O_9PSSi_2+H]^+$ 1137.3944; Found 1137.3969 (2.1 ppm error).

Compound (S_P)-10



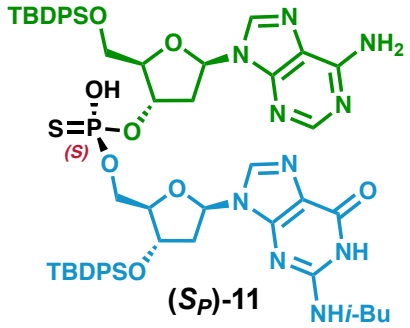
(S_P)-10

To a 50 mL flask were added compound **(R_P)-4** (1.01 g, 1.37 mmol, 1.0 equiv.) and nucleoside **SI-6** (1.15 g, 2.39 mmol, 1.8 equiv.) in THF (10 mL). DBU (0.55 mL, 3.70 mmol, 2.7 equiv.) was then added dropwise and the reaction was left to stir at ambient temperature. After 10 minutes UPLC analysis showed complete consumption of compound **(R_P)-4**. The reaction mixture was diluted with EtOAc (10 mL), DCM (5 mL), and 20% citric acid (5 mL). The organic phase was washed with brine (5 mL), dried over Na_2SO_4, filtered, and the solvent was removed *in vacuo*. The crude residue was purified by silica gel column chromatography (0 to 100% MeOH in DCM) to afford the product **(S_P)-10** as a white solid (0.88 g, 61 %).

Physical State: White solid;
^{1}H NMR (400 MHz, DMSO-d_6): δ 11.24 (s, 1H), 8.29 (s, 1H), 8.14 (s, 1H), 7.67 - 7.58 (m, 1H), 7.58 - 7.48 (m, 9H), 7.43 - 7.23 (m, 12H), 6.36 - 6.22 (m, 2H), 5.12 (br d, J=2.8 Hz, 1H), 4.48 - 4.33 (m, 1H), 4.09 - 3.90 (m, 2H), 3.81 (br dd, J=11.1, 4.3 Hz, 1H), 3.76 - 3.51 (m, 3H), 2.79-2.83 (m, 1H), 2.71 (d, J=15.4 Hz, 1H), 2.61 (d, J=15.4 Hz, 1H), 2.03 - 1.99 (m, 1H), 1.72 (s, 3H), 0.97 (s, 9H), 0.89 (s, 9H);

^{13}C NMR (101 MHz, DMSO-d_6): δ 174.5, 171.2, 163.6, 150.5, 148.5, 135.8, 135.2, 135.1, 135.0, 135.0, 134.4, 132.7, 132.6, 132.4, 130.0, 129.8, 129.8, 128.0, 127.9, 127.8, 127.7, 127.5, 119.0, 110.0, 85.7, 83.9, 83.7, 74.3, 72.4, 48.6, 42.6, 40.9, 40.8, 40.7, 40.4, 26.7, 26.6, 18.7, 18.6, 14.1, 12.1;
^{31}P NMR (162 MHz, DMSO-d_6): δ 57.0;
HRMS (ESI-TOF, m/z): Calcd for $[C_{52}H_{62}N_7O_9PSSi_2+H]^+$ 1048.3679; Found 1048.3705 (2.5 ppm error).

Compound (S_P)-11

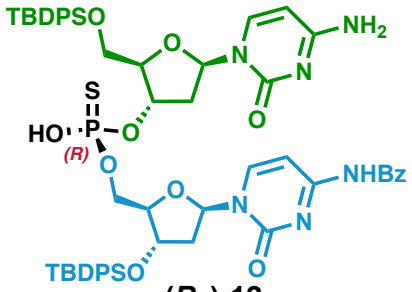


(S_P)-11

To a 50 mL flask were added compound **(R_P)-4** (803 mg, 1.09 mmol, 1.0 equiv.) and nucleoside **SI-8** (1.24 g, 2.18 mmol, 2.0 equiv.) in THF (10 mL). DBU (0.47 mL, 3.27 mmol, 3 equiv.) was then added dropwise and the reaction was left to stir at ambient temperature. After 30 minutes UPLC analysis showed complete consumption of compound **(R_P)-4**. The reaction mixture was diluted with EtOAc (20 mL) and 20% citric acid (10 mL). Then the organic phase was washed with brine (5 mL), dried over Na_2SO_4, filtered, and the solvent was removed *in vacuo*. The crude residue was purified by silica gel column chromatography (0 to 20% MeOH in DCM) to afford the product **(S_P)-11** as a white solid (986 mg, 79 %).

Physical State: White solid;
^{1}H NMR (400 MHz, DMSO-d_6): δ 12.22 (s, 1H), 12.08 (s, 1H), 8.30 (s, 1H), 8.20 (s, 1H), 8.08 (s, 1H), 7.80-7.21 (br m, 23H), 6.37 (dd, J=9.4, 5.3 Hz, 1H), 6.27 (dd, J=8.1, 6.1 Hz, 1H), 5.21-5.09 (br m, 1H), 4.75 (br d, J=4.6 Hz, 1H), 4.19-4.05 (br, 2H), 3.95-3.69 (br m, 4H), 3.08-2.93 (br m, 1H), 2.92-2.77 (m, 2H), 2.17 (br dd, J=12.6, 5.3 Hz, 1H), 1.10 (d, J=6.8 Hz, 3H), 1.07 (s, 9H), 1.05 (d, J=6.8 Hz, 3H), 0.95 (s, 9H);
^{13}C NMR (101 MHz, DMSO-d_6): δ 180.3, 155.6, 154.9, 152.0, 149.1, 148.5, 147.6, 139.1, 139.0, 135.3, 135.2, 135.1, 135.0, 133.0, 132.8, 132.7, 132.5, 130.0, 129.8, 129.7, 128.0, 127.9, 127.8, 127.7, 120.8, 119.1, 87.0, 86.9, 85.7, 85.6, 84.7, 83.5, 83.4, 75.0, 74.7, 74.6, 64.6, 64.5, 64.0, 37.7, 34.5, 26.8, 26.6, 18.9, 18.7, 18.7, 18.6;
^{31}P NMR (162 MHz, DMSO-d_6): δ 54.3;
HRMS (ESI-TOF, m/z): Calcd for $[C_{56}H_{67}N_{10}O_9PSSi_2+H]^+$ 1143.4162, Found 1143.4186 (2.1 ppm error).

Compound (R_P)-12

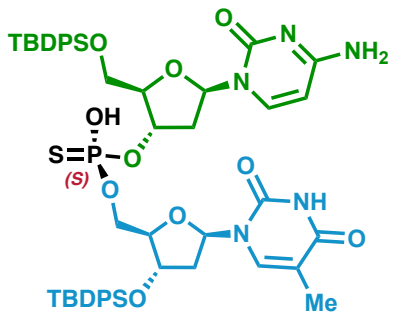


(R_P)-12

To a 50 mL flask were added compound **(S_P)-5** (1.02 g, 1.43 mmol, 1.0 equiv.) and nucleoside **SI-7** (1.60 g, 2.86 mmol, 2.0 equiv.) in MeCN (20 mL) and THF (5 mL). DBU (0.32 mL, 2.15 mmol, 3 equiv.) was then added dropwise and the reaction was left to stir at ambient temperature. After 1 hour UPLC analysis showed complete consumption of compound **SI-5**. The reaction mixture was diluted with EtOAc (25 mL) and 20% citric acid (20 mL). Then the organic phase was washed with brine (10 mL), dried over Na_2SO_4, filtered, and the solvent was removed *in vacuo*. The crude residue was purified by silica gel column chromatography (20% MeOH in DCM) to afford the product **(R_P)-12** as a white solid (1.23 g, 73%).

Physical State: White solid;
^{1}H NMR (500 MHz, DMSO-d_6): δ 11.20 (br s, 1H), 9.23 (br s, 1H), 8.47 (br d, J=7.5 Hz, 1H), 8.21 (br s, 1H), 7.98 (d, J=7.8 Hz, 2H), 7.90 (d, J=7.8 Hz, 1H), 7.64 - 7.58 (m, 9H), 7.52 - 7.37 (m, 14H), 7.29 (br s, 1H), 6.33 (t, J=7.0 Hz, 1H), 6.08 (t, J=6.5 Hz, 1H), 5.81 (d, J=7.8 Hz, 1H), 4.96 (br d, J=2.9 Hz, 1H), 4.52 - 4.45 (m, 1H), 4.16 (br s, 2H), 3.92 - 3.85 (m, 2H), 3.85 - 3.71 (m, 1H), 3.70 - 3.25 (m, 2H), 2.48 - 2.28 (m, 2H), 2.14 (dt, J=13.7, 6.7 Hz, 1H), 2.00 - 1.90 (m, 1H), 1.05(s, 9H), 0.96 (s, 9H);
^{13}C NMR (126 MHz, DMSO-d_6): δ 162.9, 159.8, 148.0, 145.2, 143.0, 135.2, 135.2, 135.1, 134.9, 134.4, 133.1, 132.7, 132.7, 132.6, 132.1, 129.9, 129.1, 128.1, 127.9, 127.5, 96.4, 93.9, 86.7, 86.1, 86.3, 85.7, 74.3, 74.2, 64.5, 63.7, 41.1, 26.7, 26.6, 18.7, 18.6;
^{31}P NMR (202 MHz, DMSO-d_6): δ 54.6;
HRMS (ESI-TOF, m/z): Calcd for $[C_{57}H_{65}N_6O_{10}PSSi_2+H]^+$ 1113.3832; Found 1113.3859 (2.4 ppm error).

Compound (S_P)-13



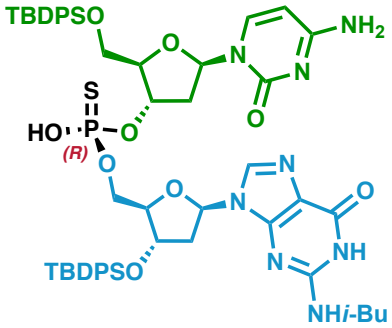
(S_P)-13

To a 50 mL flask were added compound **(R_P)-5** (1.00 g, 1.40 mmol, 1.0 equiv.) and nucleoside **SI-6** (1.35 g, 2.80 mmol, 2.0 equiv.) in MeCN (20 mL) and THF (5 mL). DBU (0.33 mL, 2.10 mmol,

3 equiv.) was then added dropwise and the reaction was left to stir at ambient temperature. After 1 hour UPLC analysis showed complete consumption of compound **(R_P)-5**. The reaction mixture was diluted with EtOAc (25 mL) and 20% citric acid (20 mL). Then the organic phase was washed with brine (10 mL), dried over Na_2SO_4, filtered and the solvent was removed *in vacuo*. The crude residue was purified by silica gel column chromatography (20% MeOH in DCM) to afford **(S_P)-13** as a white solid (1.12 g, 76 %).

Physical State: White solid;
^{1}H NMR (500 MHz, DMSO-d_6): δ 11.25 (s, 1H), 8.36 (br s, 1H), 7.84 - 7.71 (m, 3H), 7.63 (br t, J=6.3 Hz, 4H), 7.57 (br s, 4H), 7.48 - 7.36 (m, 12H), 6.36 (br t, J=7.2 Hz, 1H), 6.08 (br t, J=6.4 Hz, 1H), 5.71 (br d, J=7.3 Hz, 1H), 4.98 (br s, 1H), 4.45 (br s, 1H), 3.95 (br d, J=17.9 Hz, 2H), 3.81 - 3.76 (m, 1H), 3.73 - 3.65 (m, 2H), 3.57 - 3.50 (m, 2H), 2.40 - 2.31 (m, 1H), 2.12 - 2.04 (m, 1H), 2.04 - 1.92 (m, 2H), 1.81 (s, 3H), 1.02 (s, 9H), 0.97 (s, 9H);
^{13}C NMR (126 MHz, DMSO-d_6): δ 164.2, 162.9, 151.6, 151.0, 142.2, 136.5, 135.7, 135.6, 135.4, 133.2, 133.1, 132.6, 130.5, 130.4, 128.4, 110.6, 94.3, 86.5, 86.0, 85.7, 84.3, 75.3, 74.4, 72.9, 65.0, 64.2, 27.2, 27.1, 19.2, 19.0, 12.5;
^{31}P NMR (202 MHz, DMSO-d_6): δ 53.5;
HRMS (ESI-TOF, m/z): Calcd for $[C_{51}H_{62}N_5O_{10}PSSi_2+H]^+$ 1024.3566, Found 1024.3600 (3.3 ppm error).

Compound (R_P)-14



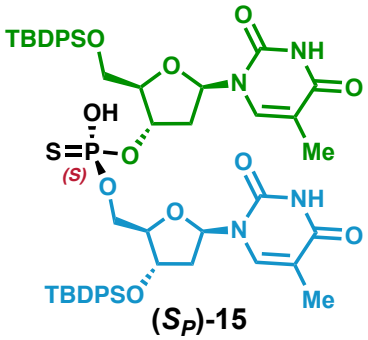
(R_P)-14

A mixture of compound **(S_P)-5** (1.00 g, 1.35 mmol, 1.00 equiv.) and the nucleoside **SI-8** (1.55 g, 2.70 mmol, 2.0 equiv.) was dissolved in a mixture of THF (10 mL) and MeCN (20 mL), then concentrated *in vacuo* (3x). The residue was dissolved in a mixture of THF (10 mL) and MeCN (20 mL) and DBU (608 µL, 4.04 mmol, 3.0 equiv.) was added. The reaction mixture was stirred for 30 min, then diluted with EtOAc (20 mL) and aqueous 1 N HCl (20 mL, pH = 1). The layers were separated and the aqueous layer was extracted with EtOAc (2 x 20 mL). The combined organic extracts were dried over sodium sulfate, filtered, and concentrated *in vacuo* to afford a gel. The gel was purified by flash column chromatography (5 to 20% MeOH in DCM, performed twice). The desired product **(R_P)-14** was isolated as a gel, which was stirred in MTBE (40 mL) for 1 h to convert the product to a white powder which was isolated by filtration (1.23 g, 82%).

Physical State: White solid;
^{1}H NMR (400 MHz, DMSO-d_6): δ 12.31 (br s, 1H), 12.08 (br s, 1H), 9.23 (br s, 1H), 8.25 (br s, 2H), 7.90 (d, J = 7.8 Hz, 1H), 7.53-7.70 (m, 9H), 7.35-7.49 (m, 13H), 6.35 (dd, J = 9.5, 5.4 Hz, 1H), 6.04 (t, J = 6.5 Hz, 1H), 5.80 (d, J = 7.8 Hz, 1H), 4.94 (dt, J = 6.0, 2.9 Hz, 1H), 4.57 (br d, J

= 4.5 Hz, 1H), 4.08-4.11 (m, 2H), 3.75-3.93 (m, 3H), 3.53-3.58 (m, 3H), 2.76-2.96 (m, 2H), 2.24-2.33 (m, 1H), 2.26-2.20 (m, 2H), 1.05-1.10 (m, 16H), 0.97 (s, 9H);
^{13}C NMR (101 MHz, DMSO-d_6): δ 180.4, 159.7, 154.9, 148.5, 147.86, 147.82, 143.1, 138.5, 135.25, 135.20, 135.1, 134.9, 132.74, 132.70, 132.6, 129.98, 129.94, 129.90, 127.94, 127.88, 120.6, 94.0, 86.60, 86.53, 86.13, 85.8, 84.3, 74.7, 74.2, 64.8, 63.7, 34.6, 26.7, 26.6, 18.8, 18.7, 18.6;
^{31}P NMR (162 MHz, DMSO-d_6): δ 54.78;
HRMS (ESI-TOF, m/z): Calcd for $[C_{55}H_{67}N_8O_{10}PSSi_2+H]^+$ 1119.4050; Found 1119.4071 (1.9 ppm error).

Compound (S_P)-15

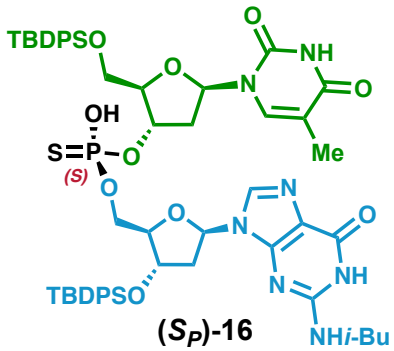


(S_P)-15

To a solution of the compound **(R_P)-6** (1.00 g, 1.26 mmol, 1.0 equiv.) and nucleoside **SI-6** (1.17 g, 1.17 g, 2.52 mmol, 2.0 equiv.) in THF (10 mL) was added DBU (581 µL, 3.78 mmol, 3.0 equiv.). The reaction mixture was stirred for 30 min, then diluted with EtOAc (10 mL) and aqueous 1 N HCl (5 mL, pH = 1). The layers were separated and the aqueous layer was extracted with EtOAc (10 mL). The combined organic extracts were dried over Na_2SO_4, filtered, then concentrated *in vacuo* to afford a gel. The gel was purified by flash column chromatography (0 to 10% to 30% MeOH in DCM to 30%, performed twice). The desired product **(S_P)-15** was isolated as a gel, which was concentrated from hexanes (10 mL) to afford a white powder (926.1 mg, 72%).

Physical State: White solid;
^{1}H NMR (400 MHz, DMSO-d_6): δ 11.37 (s, 1H), 11.26 (s, 1H), 7.84 (s, 1H), 7.61-7.66 (m, 5H), 7.55-7.57 (m, 4H), 7.35-7.45 (m, 13H), 6.35 (dd, J = 8.5, 6.2 Hz, 1H), 6.13 (dd, J = 8.8, 5.6 Hz, 1H), 5.03-5.07 (m, 1H), 4.40 (m, 1H), 3.94 (br d, J = 9.3 Hz, 2H), 3.65-3.76 (m, 3H), 3.41-3.53 (m, 1H), 2.24 (br dd, J = 12.9, 5.1 Hz, 1H), 2.03-2.14 (m, 1H), 1.90-2.03 (m, 2H), 1.82 (s, 3H), 1.43 (s, 3H), 1.01 (s, 9H), 0.98 (s, 9H);
^{13}C NMR (101 MHz, DMSO-d_6): δ 163.8, 163.6, 150.6, 150.4, 136.1, 135.20, 135.18, 135.1, 135.0, 134.9, 132.8, 132.72, 132.68, 132.0, 130.03, 129.96, 127.96, 127.91, 110.2, 109.7, 86.2, 86.1, 85.4, 85.3, 83.9, 74.97, 74.85, 64.4, 64.2, 38.7, 26.7, 26.6, 18.8, 18.5, 12.1, 11.8;
^{31}P NMR (162 MHz, DMSO-d_6): δ 53.01;
HRMS (ESI-TOF, m/z): Calcd for $[C_{52}H_{63}N_4O_{11}PSSi_2+H]^+$ 1039.3563; Found 1039.3586 (2.3 ppm error).

Compound (S_P)-16

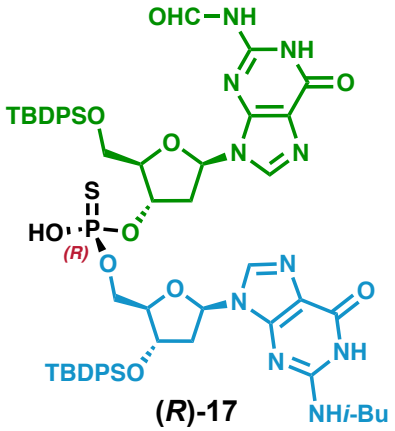


(S_P)-16

To a mixture of **(R_P)-6** (734 mg, 1.0 equiv.) and **SI-8** (1.20 g, 2.1 equiv.) in THF was added DBU (0.45 mL, 3.0 equiv.). After 30 min, EtOAc (20 mL) and 20% citric acid (10 mL) were added. The phases were separated and the organic phase was washed with brine (5 mL) and dried over Na_2SO_4. The solution was filtered and the solvent was removed under vacuum. The residue was dissolved with DCM, and purified by ISCO flash chromatography (80 g silica gel column, 0 to 20% MeOH in DCM, 18 min run). The pure fractions were combined and solvents were removed to afford the desired product **(S_P)-16** (380 mg) as white solid. Then, the impure fractions were combined, solvents were removed under vacuo and a second ISCO flash chromatography was performed using the same conditions. The desired product **(S_P)-16** (388 mg) was obtained as white solid. In total, **(S_P)-16** (768 mg, 67%) was obtained.

Physical State: White solid;
^{1}H NMR (400 MHz, DMSO-d_6): δ 12.17 (s, 1H), 12.06 (s, 1H), 11.37 (s, 1H), 8.30 (s, 1H), 7.77-7.35 (br m, 21H), 6.35 (dd, J=9.6, 5.3 Hz, 1H), 6.13 (dd, J=8.8, 5.6 Hz, 1H), 5.16-5.01 (br m, 1H), 4.71 (br d, J=4.6 Hz, 1H), 4.12-4.04 (br m, 1H), 4.02-3.93 (br m, 1H), 3.86-3.73 (br m, 3H), 3.72-3.63 (br m, 1H), 3.02-2.89 (br m, 1H), 2.87-2.76 (br m, 1H), 2.30 (br dd, J=13.1, 6.8 Hz, 1H), 2.20-2.04 (br m, 2H), 1.44 (s, 3H), 1.12 (d, J=6.8 Hz, 3H), 1.10 (d, J=6.8 Hz, 3H), 1.06 (s, 9H), 0.99 (s, 9H);
^{13}C NMR (101 MHz, DMSO-d_6): δ 180.3, 163.6, 154.9, 150.3, 148.5, 147.6, 139.1, 135.3, 135.2, 135.1, 135.0, 134.8, 132.9, 132.8, 132.8, 132.1, 129.9, 128.0, 127.9, 120.7, 109.6, 87.0, 86.9, 85.3, 85.2, 84.6, 83.8, 75.0, 74.7, 74.6, 64.3, 64.2, 64.1, 54.9, 38.7, 38.4, 34.5, 26.8, 26.6, 18.9, 18.8, 18.7, 18.6, 11.7;
^{31}P NMR (162 MHz, DMSO-d_6): δ 54.1;
HRMS (ESI-TOF, m/z): Calcd for [C56H68N7O11PSSi2+H]+ 1134.4046, Found 1134.4080 (2.9 ppm error).

Compound (R_P)-17

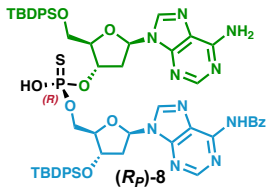
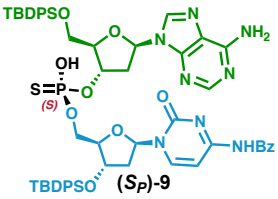
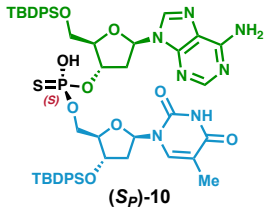
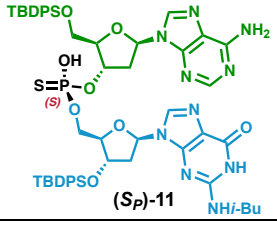
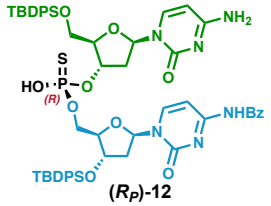
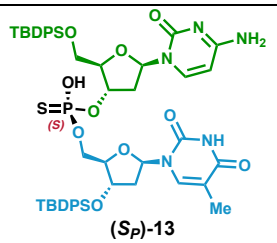


(*R*)-17

To a 50 mL flask were added compound **(S_P)-7** (1.00 g, 1.24 mmol, 1.0 equiv.) and nucleoside **SI-8** (1.42 g, 2.48 mmol, 2.0 equiv.) in MeCN (20 mL) and THF (3 mL). DBU (0.37 mL, 2.48 mmol, 2 equiv.) was then added dropwise and the reaction was left to stir at ambient temperature. After 2 hour UPLC analysis showed complete consumption of compound **(*S*)-7**. The reaction mixture was diluted with EtOAc (25 mL) and 20% citric acid (25 mL) and was allowed to stir at ambient temperature for 4 hours. The dimethylaminomethylene was converted to formamide based on HPLC-MS analysis. Then the organic phase was washed with brine (25 mL), dried over Na_2SO_4, filtered and the solvent was removed *in vacuo*. The crude residue was purified by silica gel column chromatography (0 to 60% MeOH in EtOAc) to afford the product **(R_P)-17** as a white solid (1.27 g, 86%).

Physical State: White solid;
^{1}H NMR (400 MHz, DMSO-d_6): δ 12.33 (br s, 1H), 12.10 (s, 1H), 8.28 (s, 1H), 8.00 (s, 1H), 7.78 - 7.63 (m, 1H), 7.63 - 7.54 (m, 8H), 7.46 - 7.25 (m, 13H), 6.36 (dd, J=9.2, 5.4 Hz, 1H), 6.14 (t, J=6.6 Hz, 1H), 5.05 (br s, 1H), 4.64 - 4.55 (m, 1H), 4.14 (br s, 2H), 4.00 - 3.88 (m, 1H), 3.88 - 3.70 (m, 2H), 3.67 - 3.55 (m, 1H), 2.96 - 2.74 (m, 2H), 2.58 - 2.50 (m, 1H), 2.33 - 2.14 (m, 2H), 1.12 - 1.05 (m, 6H), 1.02 (s, 9H), 0.93 (s, 9H);
^{13}C NMR (101 MHz, DMSO-d_6): δ δ 180.5, 154.9, 148.5, 148.3, 147.8, 147.2, 139.1, 137.3, 135.2, 135.2, 135.1, 135.0, 132.8, 132.5, 130.0, 129.8, 129.7, 128.0, 127.9, 127.8, 127.7, 120.8, 120.6, 86.7, 86.6, 86.2, 86.2, 84.8, 83.3, 74.8, 74.6, 64.7, 64.3, 48.6, 37.9, 34.6, 26.8, 26.6, 18.9, 18.8, 18.6;
^{31}P NMR (162 MHz, DMSO-d_6): δ 54.2;
HRMS (ESI-TOF, m/z): Calcd for $[C_{57}H_{67}N_{10}O_{11}PSSi_2+H]^+$ 1187.4060; Found 1187.4085 (2.0 ppm error).

UPLC/HPLC Methods (Table S5)

Entry	Product	Assay Conditions	Retention time
1	 (R_P)-8	UPLC Ascentis express C18 2.7 um 2.1 x 50 mm Solvent **A**: 0.05% TFA in MeCN:H_2O (5:95) Solvent **B**: 0.05% TFA in MeCN:H_2O (95:5) Gradient: Complex – 0% to 100% **B** over 2 min Flow rate: 1 mL/min PDA wavelength: 220 nm	1.91
2	 (S_P)-9	UPLC Ascentis express C18 2.6 um 2.1 x 50 mm Solvent **A**: 0.05% TFA in MeCN:H_2O (5:95) Solvent **B**: 0.05% TFA in MeCN:H_2O (95:5) Gradient: Complex – 0% to 100% **B** over 2 min Flow rate: 1 mL/min PDA wavelength: 220 nm	2.08
3	 (S_P)-10	UPLC Ascentis express C18 2.7 um 2.1 x 50 mm Solvent **A**: 0.05% TFA in MeCN:H_2O (5:95) Solvent **B**: 0.05% TFA in MeCN:H_2O (95:5) Gradient: Complex – 10% to 100% **B** over 2 min Flow rate: 1 mL/min PDA wavelength: 220 nm	1.88
4	 (S_P)-11	UPLC Ascentis express C18 2.7 um 2.1 x 50 mm Solvent **A**: 0.01% NH_4OAc in MeCN:H_2O (5:95) Solvent **B**: 0.01% NH_4OAc in MeCN:H_2O (95:5) Gradient: Complex – 0% to 100% **B** over 2 min Flow rate: 1 mL/min PDA wavelength: 220 nm	1.92
5	 (R_P)-12	UPLC Thermo Accucore aQ 2.6 um 2.1 x 50 mm Solvent **A**: 0.01% NH_4OAc in MeCN:H_2O (5:95) Solvent **B**: 0.01% NH_4OAc in MeCN:H_2O (95:5) Gradient: Complex – 10% to 100% **B** over 2 min Flow rate: 1 mL/min PDA wavelength: 220 nm	1.49
6	 (S_P)-13	UPLC Ascentis express C18 2.7 um 2.1 x 50 mm Solvent **A**: 0.01% NH_4OAc in MeCN:H_2O (5:95) Solvent **B**: 0.01% NH_4OAc in MeCN:H_2O (95:5) Gradient: Complex – 10% to 100% **B** over 2 min Flow rate: 1 mL/min PDA wavelength: 220 nm	1.77

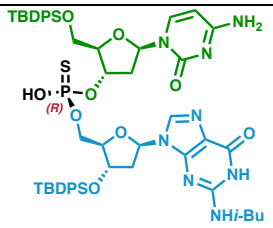
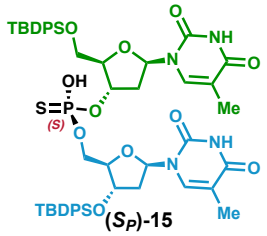
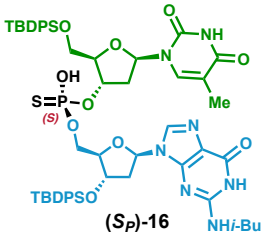
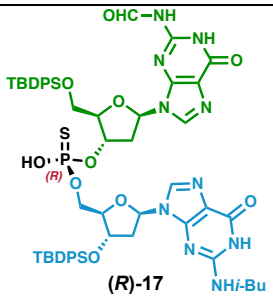
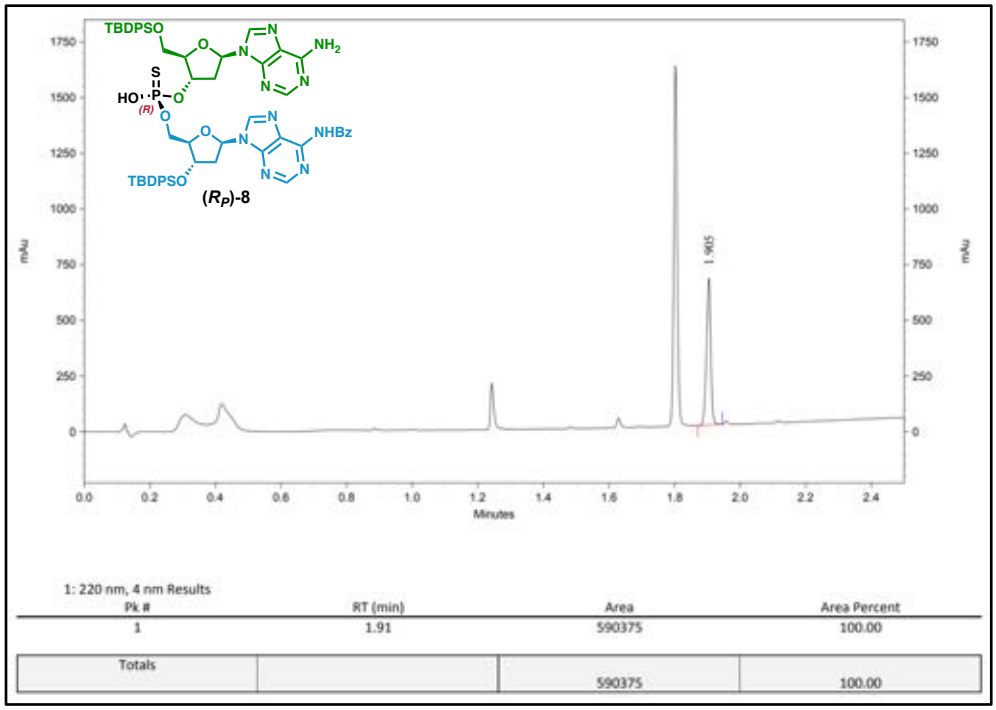
7	 (R_P)-14	HPLC Xbridge BEH Shield RP18 2.5 um 4.6 x 50 mm Solvent **A**: 0.05% TFA in MeOH:H_2O (20:80) Solvent **B**: 0.05% TFA in MeOH:ACN (20:80) Gradient: Complex –0% to 100% **B** over 30 min Flow rate: 0.8 mL/min PDA wavelength: 220 nm, 256 nm	22.91
8	 (S_P)-15	HPLC Supelco Ascentis express C18 2.7 um 4.6 x 150 mm Solvent **A**: 0.05% TFA in MeOH:H_2O (20:80) Solvent **B**: 0.05% TFA in MeOH:ACN (20:80) Gradient: Complex –0% to 100% **B** over 30 min Flow rate: 1 mL/min PDA wavelength: 220 nm, 256 nm	30.82
9	 (S_P)-16	UPLC Ascentis express C18 2.7 um 2.1 x 50 mm Solvent **A**: 0.01% NH_4OAc in MeCN:H_2O (5:95) Solvent **B**: 0.01% NH_4OAc in MeCN:H_2O (95:5) Gradient: Complex – 0% to 100% **B** over 2 min Flow rate: 1 mL/min PDA wavelength: 220 nm	2.04
10	 (*R*)-17	UPLC Agilent Poroshell EC-C18 1.9 um 2.1 x 50 mm Solvent **A**: 0.01% NH_4OAc in MeCN:H_2O (5:95) Solvent **B**: 0.01% NH_4OAc in MeCN:H_2O (95:5) Gradient: Complex – 0% to 100% **B** over 2 min Flow rate: 1 mL/min PDA wavelength: 220 nm	2.02

Table S5: UPLC/HPLC conditions and retention times for compounds **8-17**.

UPLC/HPLC Chromatograms of compounds 8-17

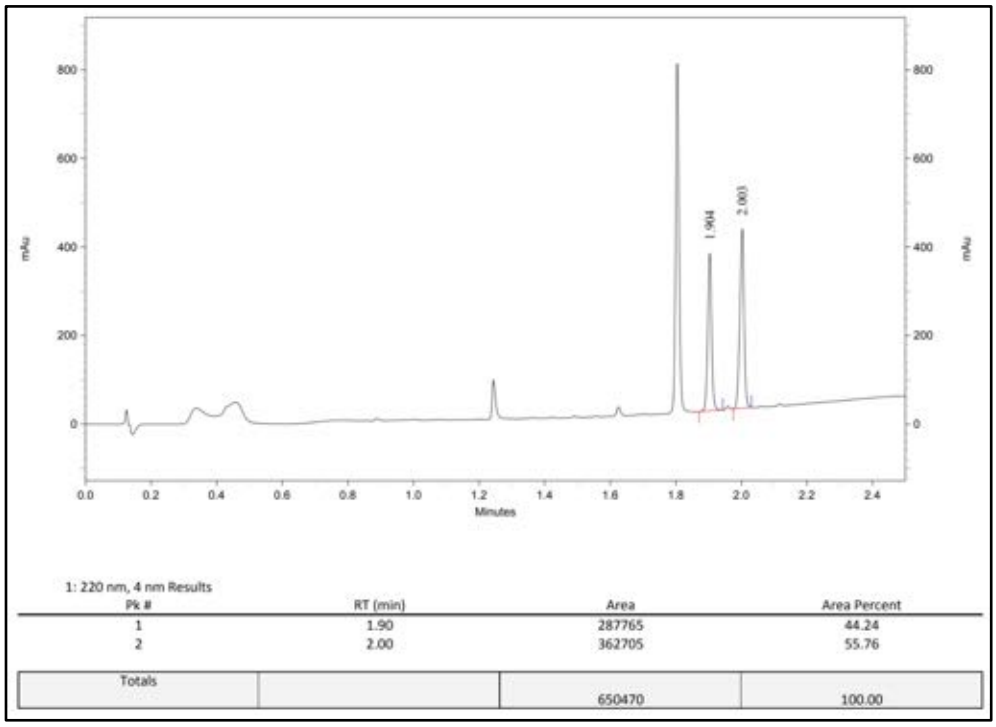
Crude mixture



1: 220 nm, 4 nm Results

Pk #	RT (min)	Area	Area Percent
1	1.91	590375	100.00
Totals		590375	100.00

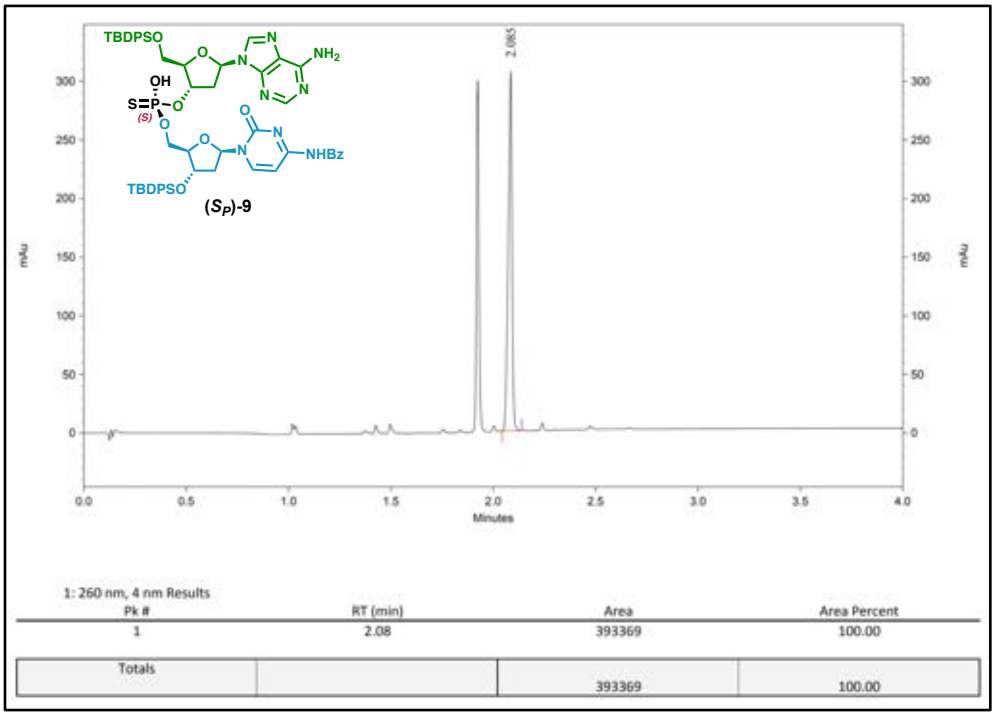
After addition of the (*S*)-diastereomer (retention time = 2.00 min)



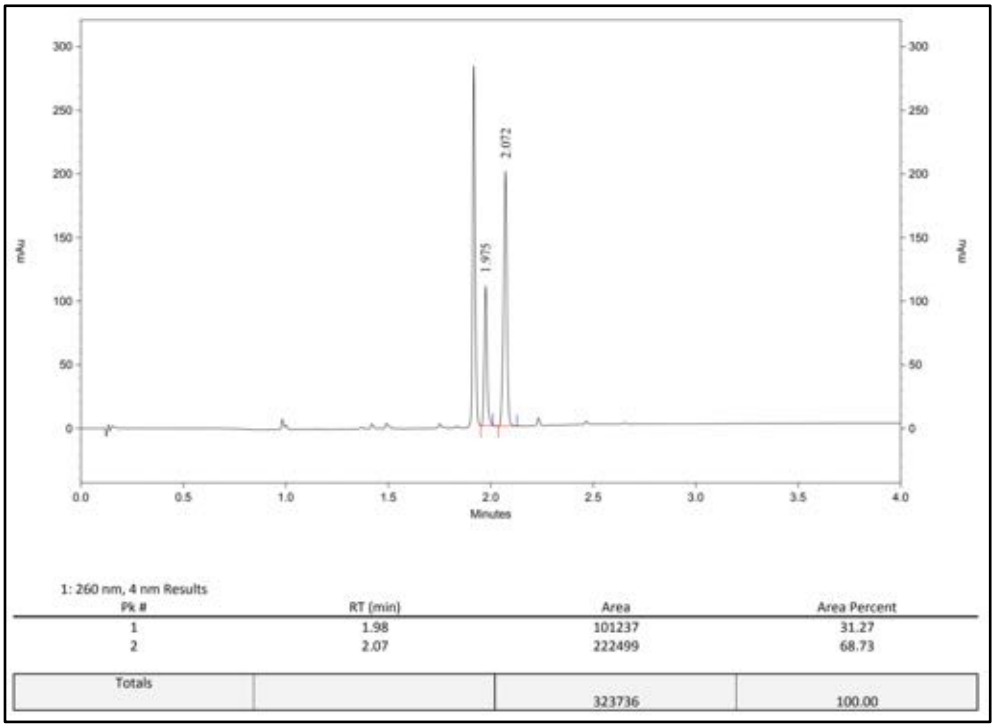
1: 220 nm, 4 nm Results

Pk #	RT (min)	Area	Area Percent
1	1.90	287765	44.24
2	2.00	362705	55.76
Totals		650470	100.00

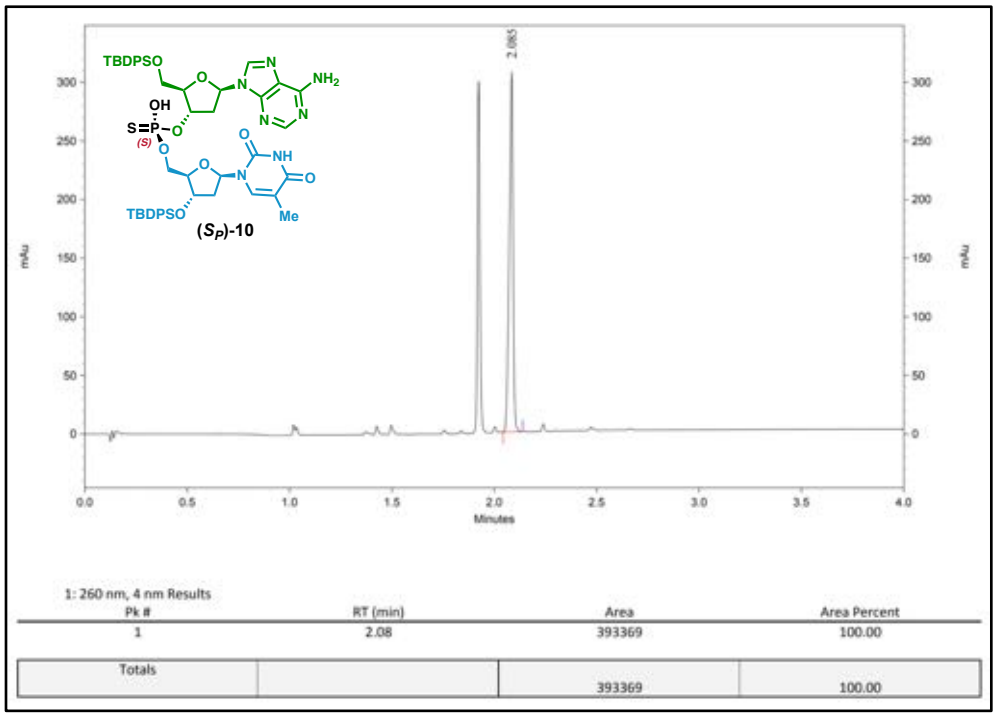
Crude mixture



After addition of the (*R*)-diastereomer (retention time = 1.98 min)

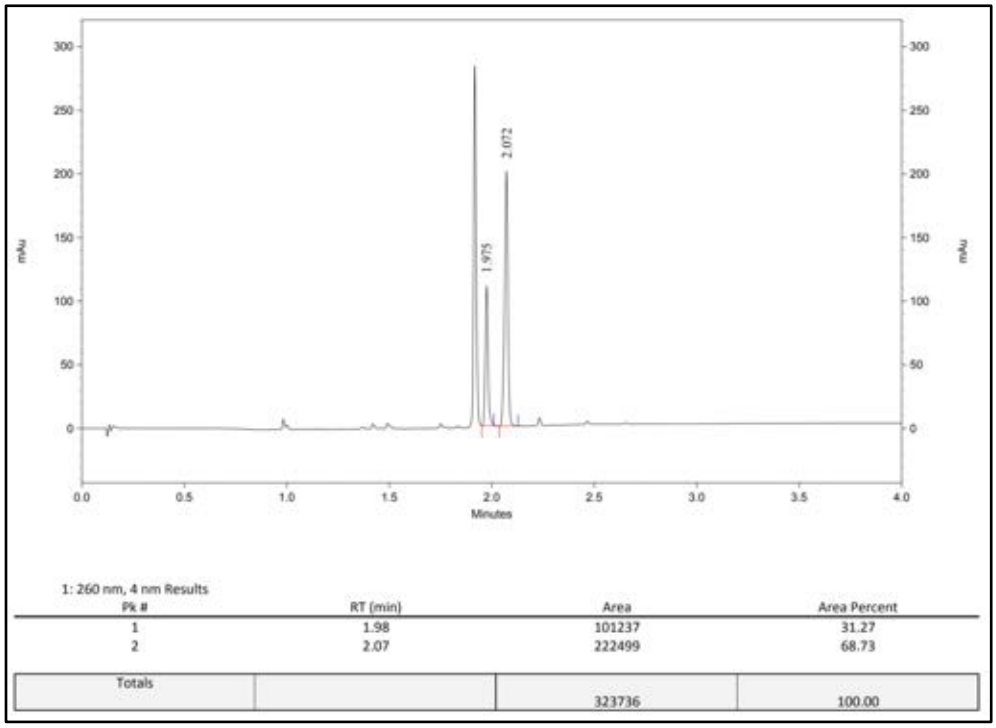


Crude mixture



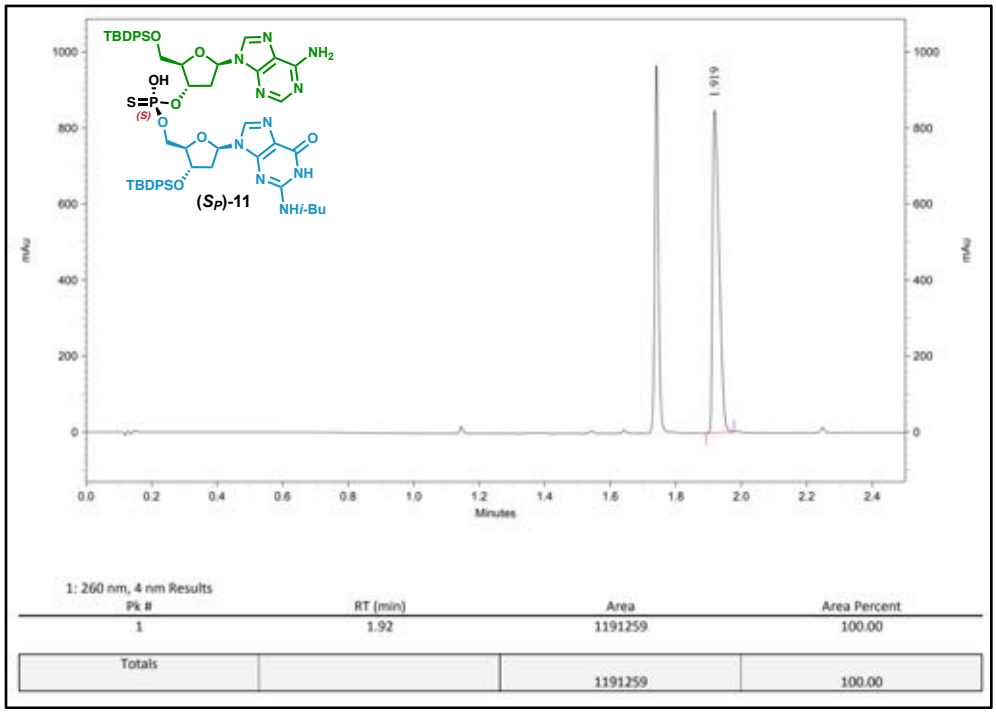
Pk #	RT (min)	Area	Area Percent
1	2.08	393369	100.00
Totals		393369	100.00

After addition of the (*R*)-diastereomer (retention time = 1.98 min)



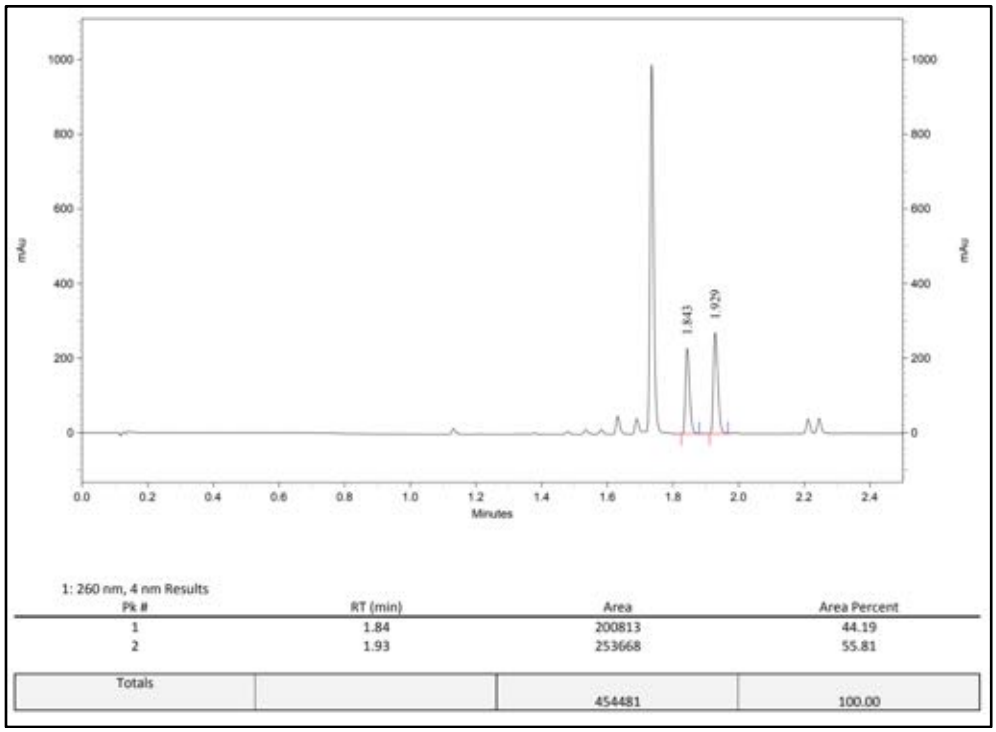
Pk #	RT (min)	Area	Area Percent
1	1.98	101237	31.27
2	2.07	222499	68.73
Totals		323736	100.00

Crude mixture



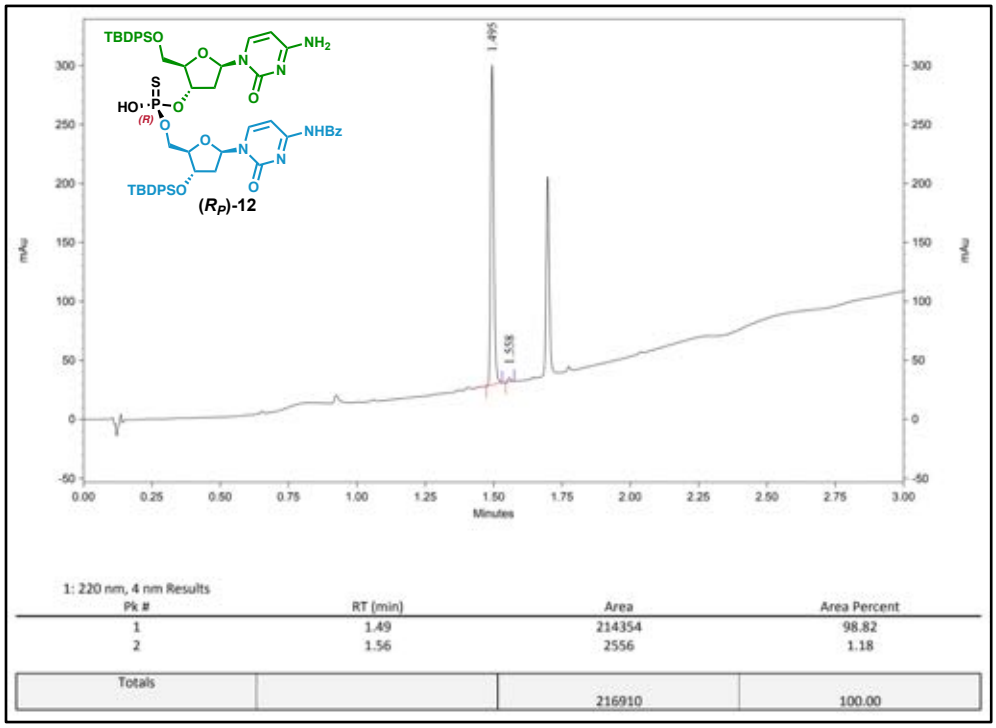
Pk #	RT (min)	Area	Area Percent
1	1.92	1191259	100.00
Totals		1191259	100.00

After addition of the (*R*)-diastereomer (retention time = 1.84 min)



Pk #	RT (min)	Area	Area Percent
1	1.84	200813	44.19
2	1.93	253668	55.81
Totals		454481	100.00

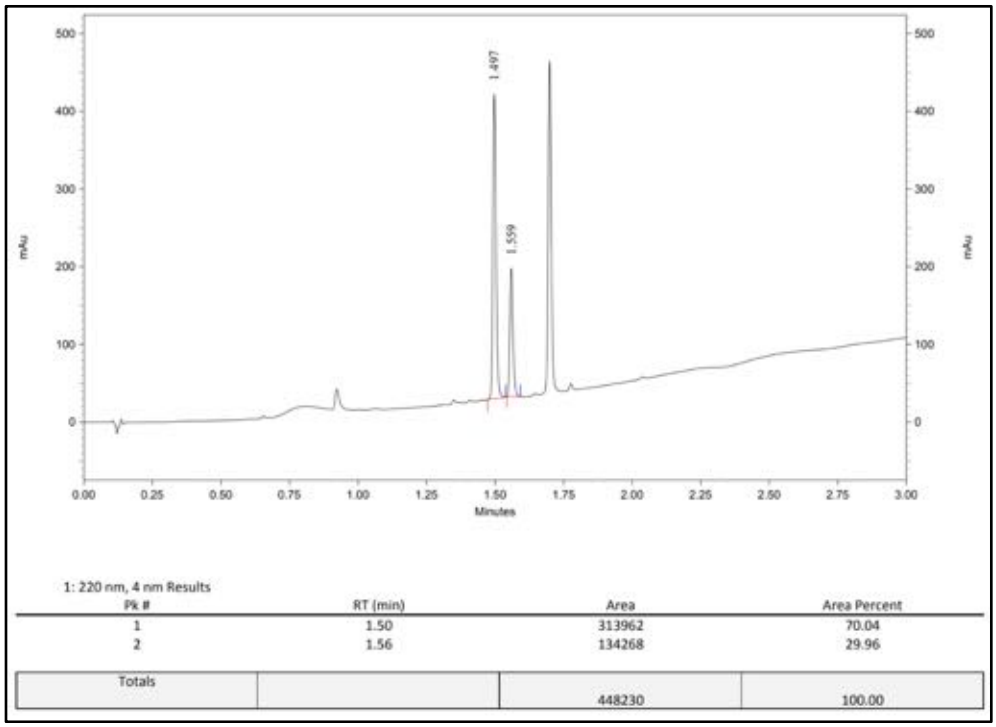
Crude mixture



1: 220 nm, 4 nm Results

Pk #	RT (min)	Area	Area Percent
1	1.49	214354	98.82
2	1.56	2556	1.18
Totals		216910	100.00

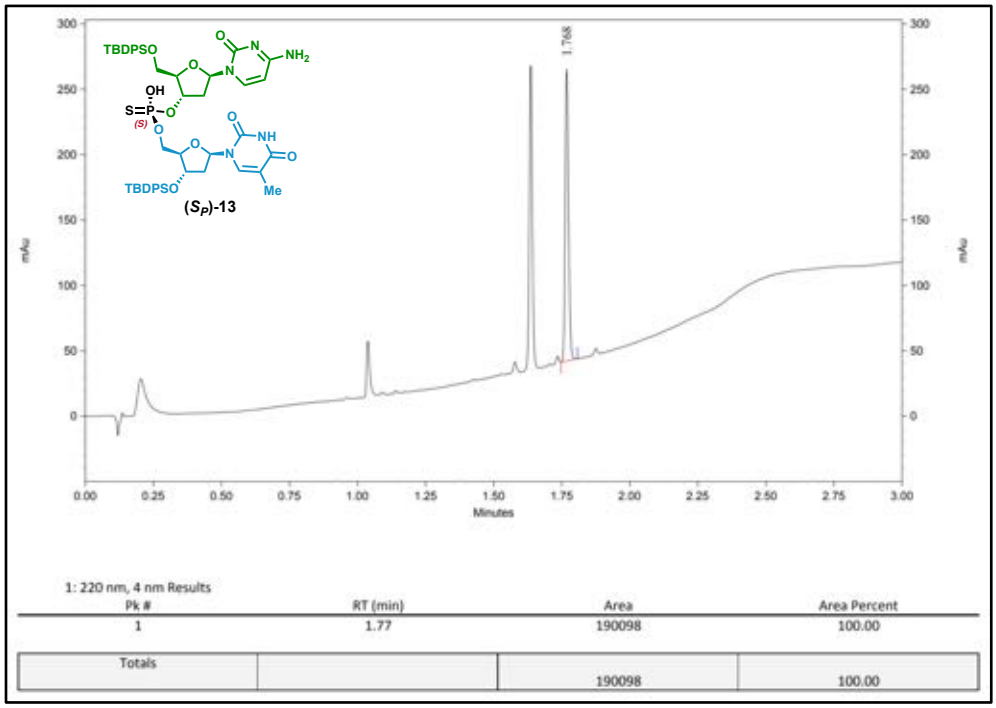
After addition of the (*S*)-diastereomer (retention time = 1.56 min)



1: 220 nm, 4 nm Results

Pk #	RT (min)	Area	Area Percent
1	1.50	313962	70.04
2	1.56	134268	29.96
Totals		448230	100.00

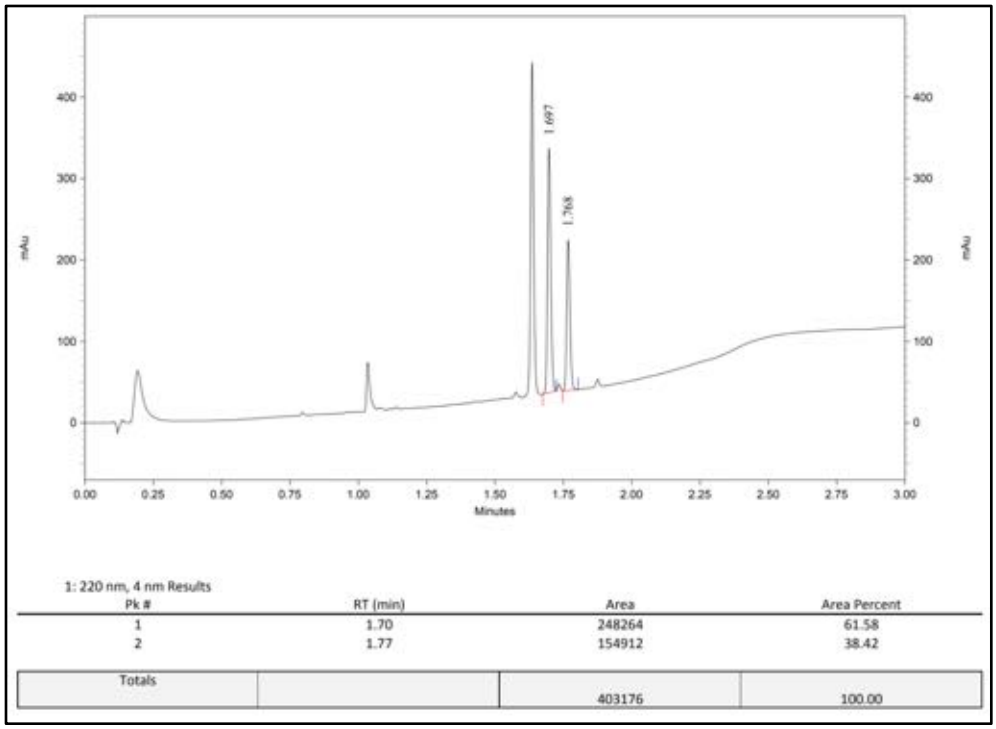
Crude mixture



1: 220 nm, 4 nm Results

Pk #	RT (min)	Area	Area Percent
1	1.77	190098	100.00
Totals		190098	100.00

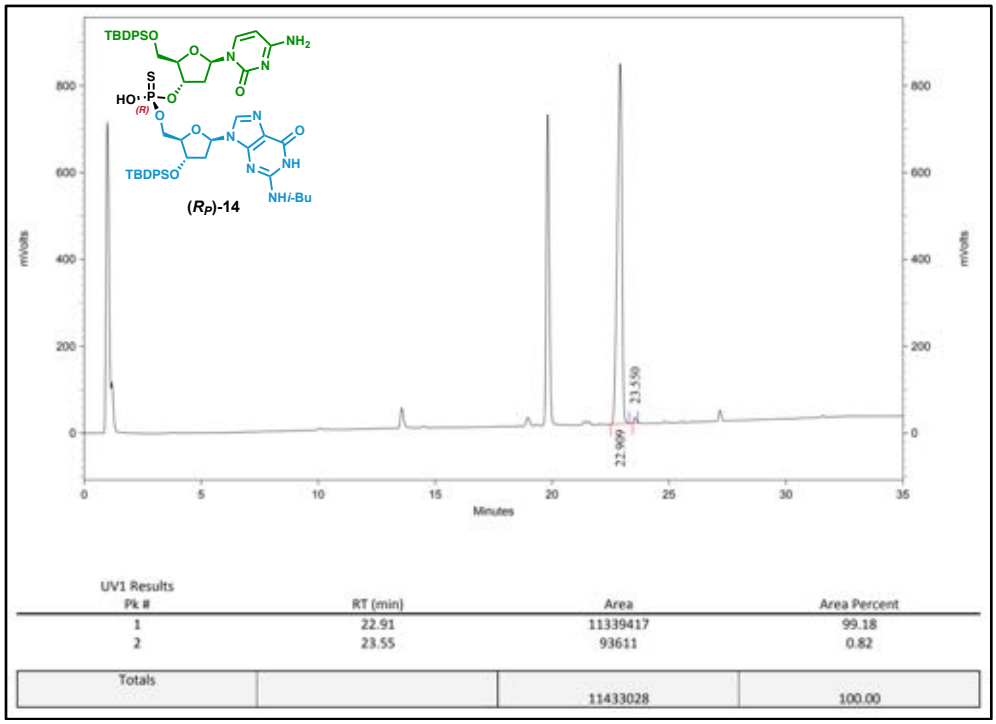
After addition of the (*R*)-diastereomer (retention time = 1.70 min)



1: 220 nm, 4 nm Results

Pk #	RT (min)	Area	Area Percent
1	1.70	248264	61.58
2	1.77	154912	38.42
Totals		403176	100.00

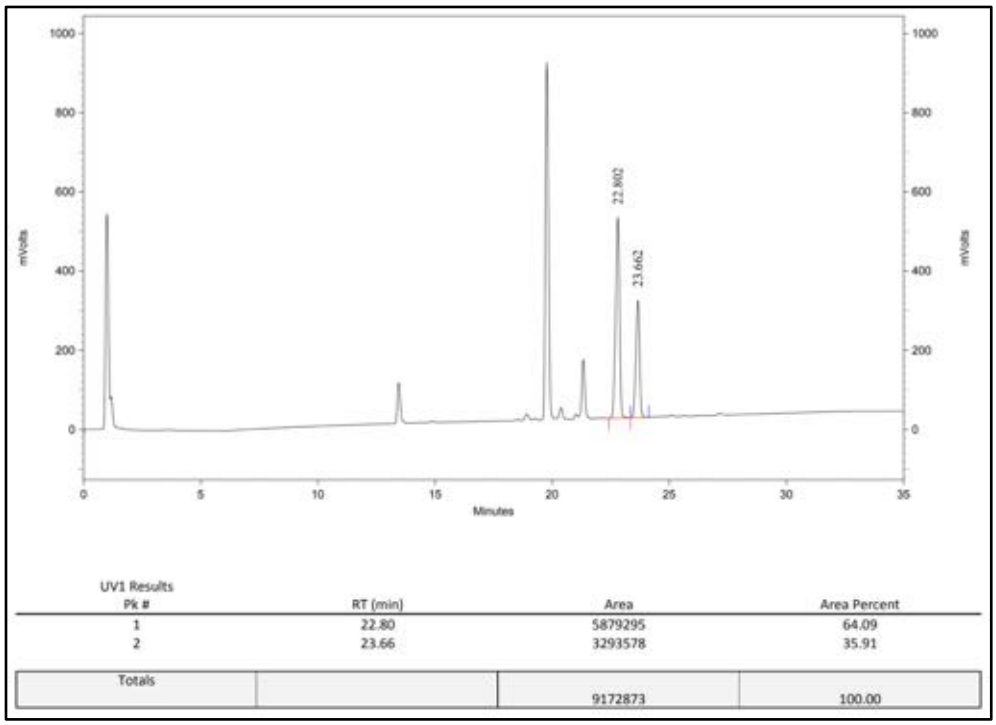
Crude mixture



UV1 Results

Pk #	RT (min)	Area	Area Percent
1	22.91	11339417	99.18
2	23.55	93611	0.82
Totals		11433028	100.00

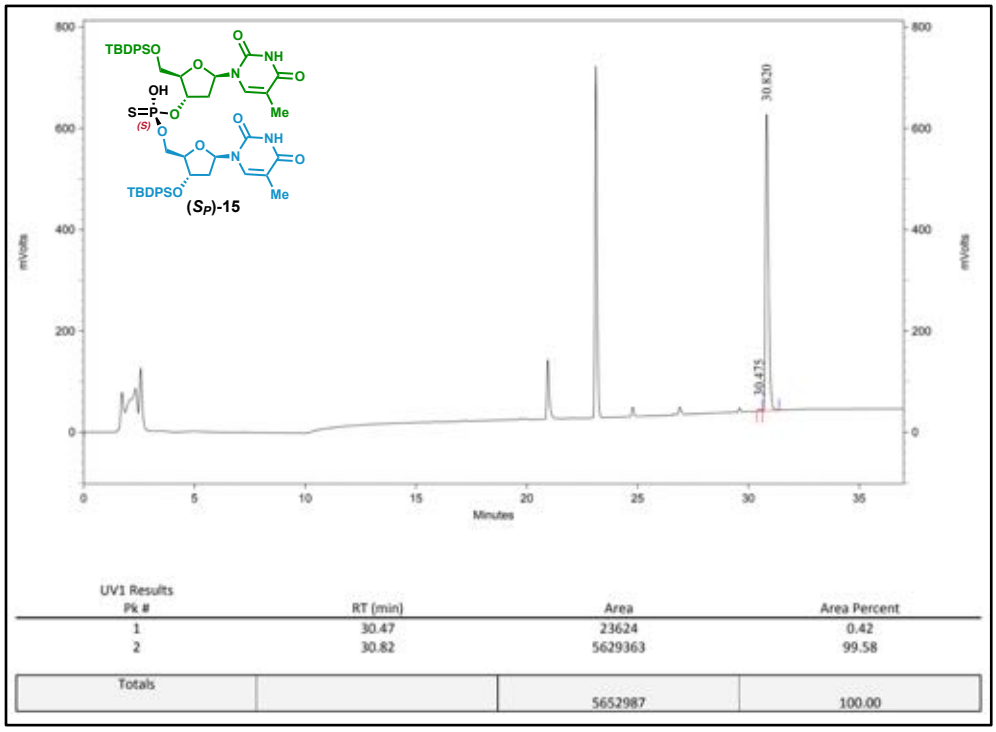
After addition of the (*S*)-diastereomer (retention time = 23.66 min)



UV1 Results

Pk #	RT (min)	Area	Area Percent
1	22.80	5879295	64.09
2	23.66	3293578	35.91
Totals		9172873	100.00

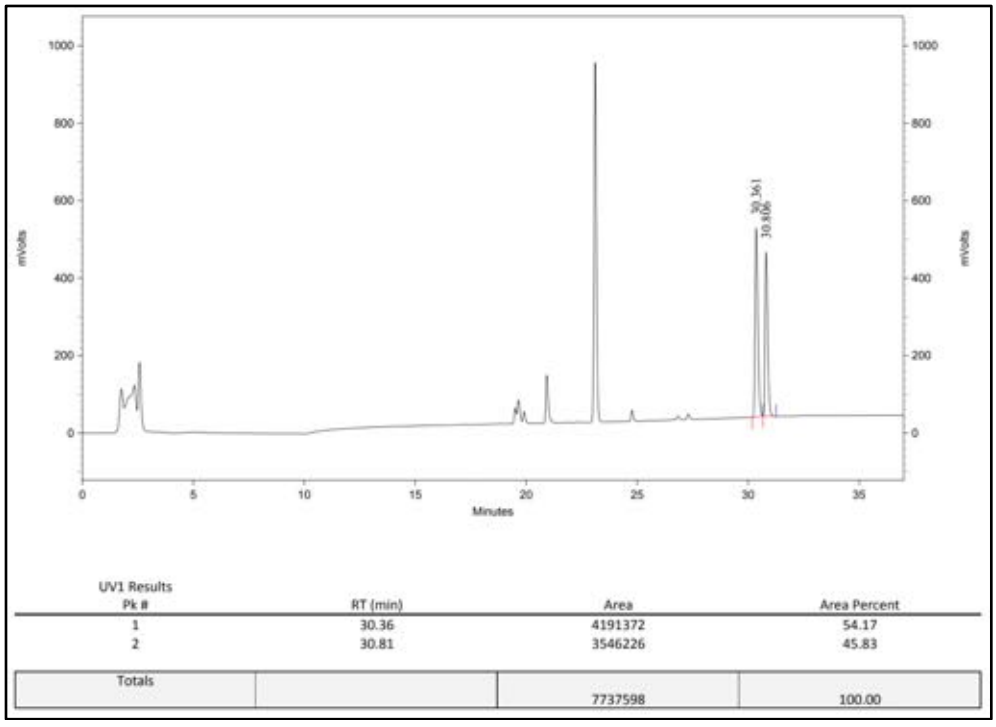
Crude mixture



UV1 Results

Pk #	RT (min)	Area	Area Percent
1	30.47	23624	0.42
2	30.82	5629363	99.58
Totals		5652987	100.00

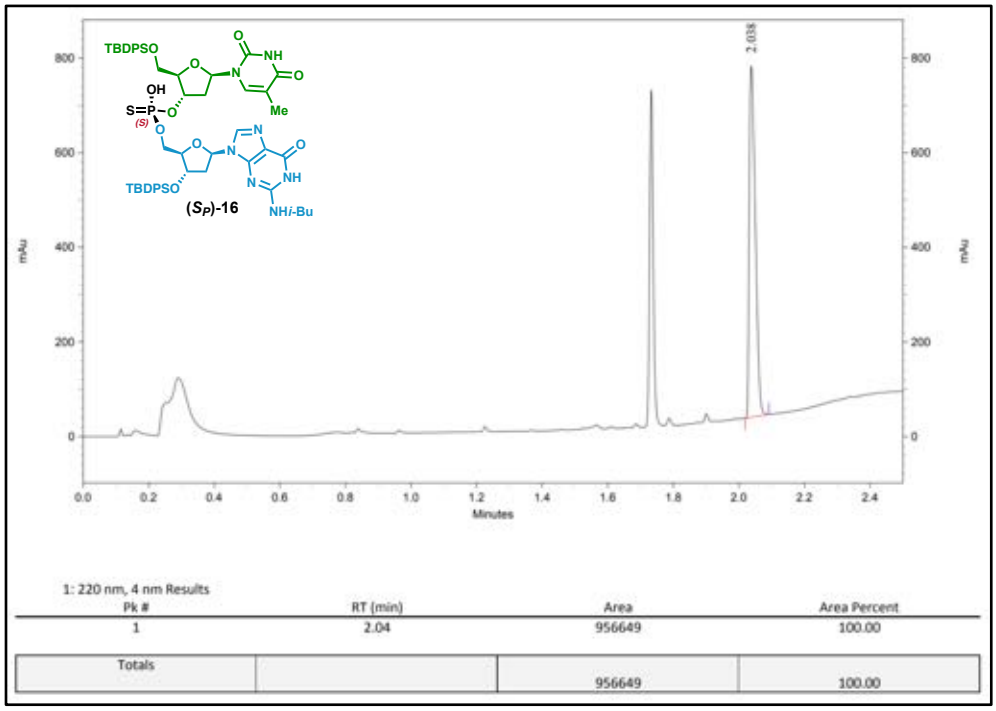
After addition of the (*R*)-diastereomer (retention time = 30.36 min)



UV1 Results

Pk #	RT (min)	Area	Area Percent
1	30.36	4191372	54.17
2	30.81	3546226	45.83
Totals		7737598	100.00

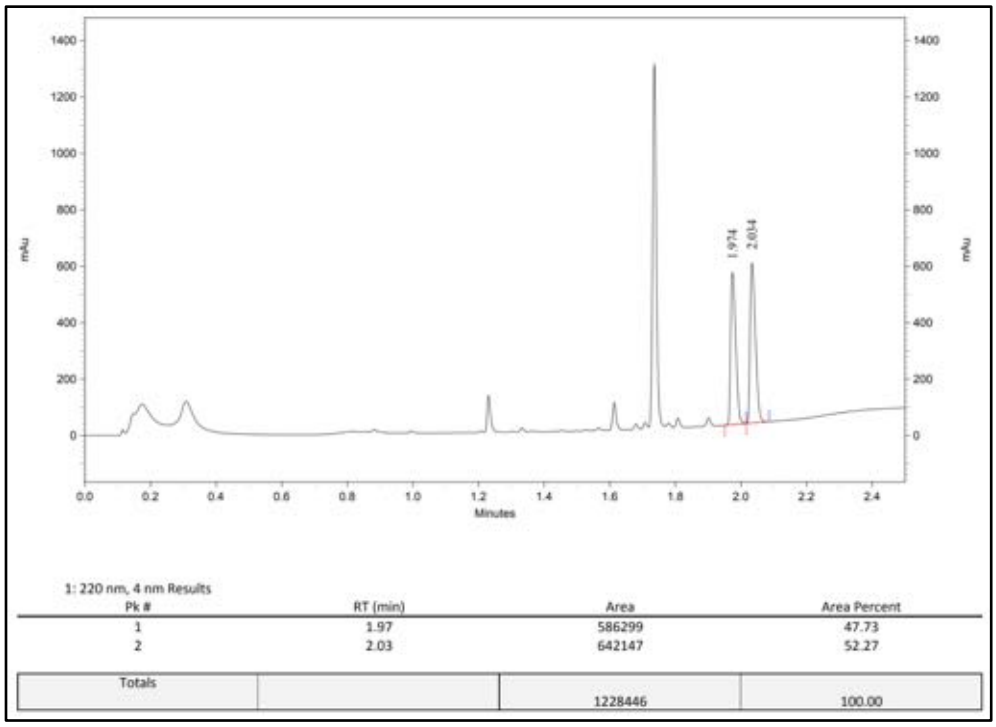
Crude mixture



1: 220 nm, 4 nm Results

Pk #	RT (min)	Area	Area Percent
1	2.04	956649	100.00
Totals		956649	100.00

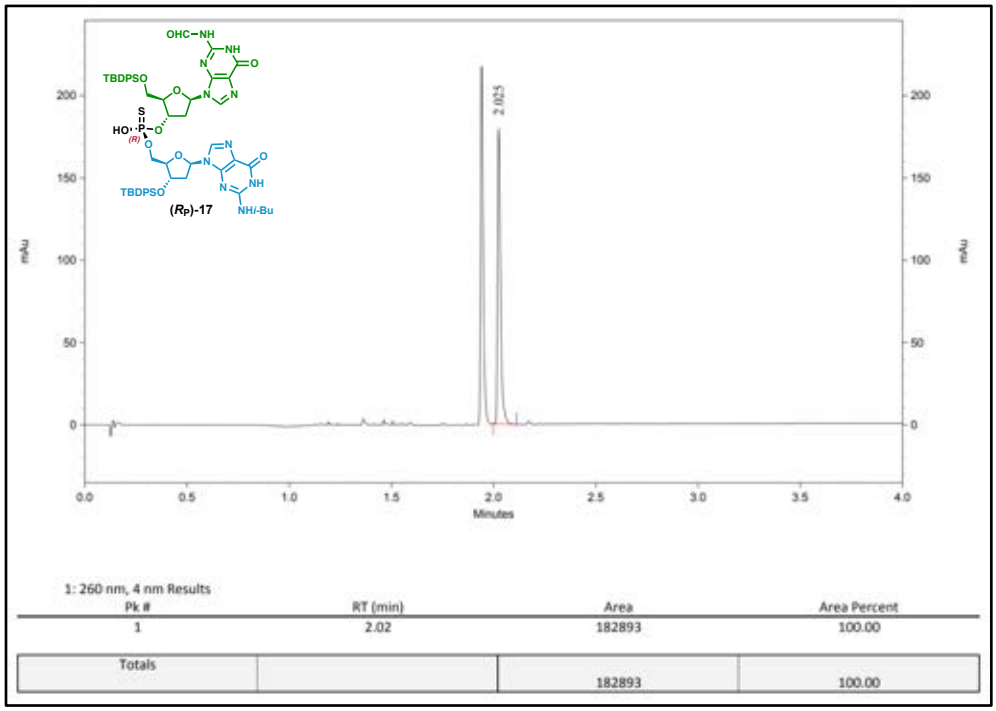
After addition of the (*R*)-diastereomer (retention time = 1.97 min)



1: 220 nm, 4 nm Results

Pk #	RT (min)	Area	Area Percent
1	1.97	586299	47.73
2	2.03	642147	52.27
Totals		1228446	100.00

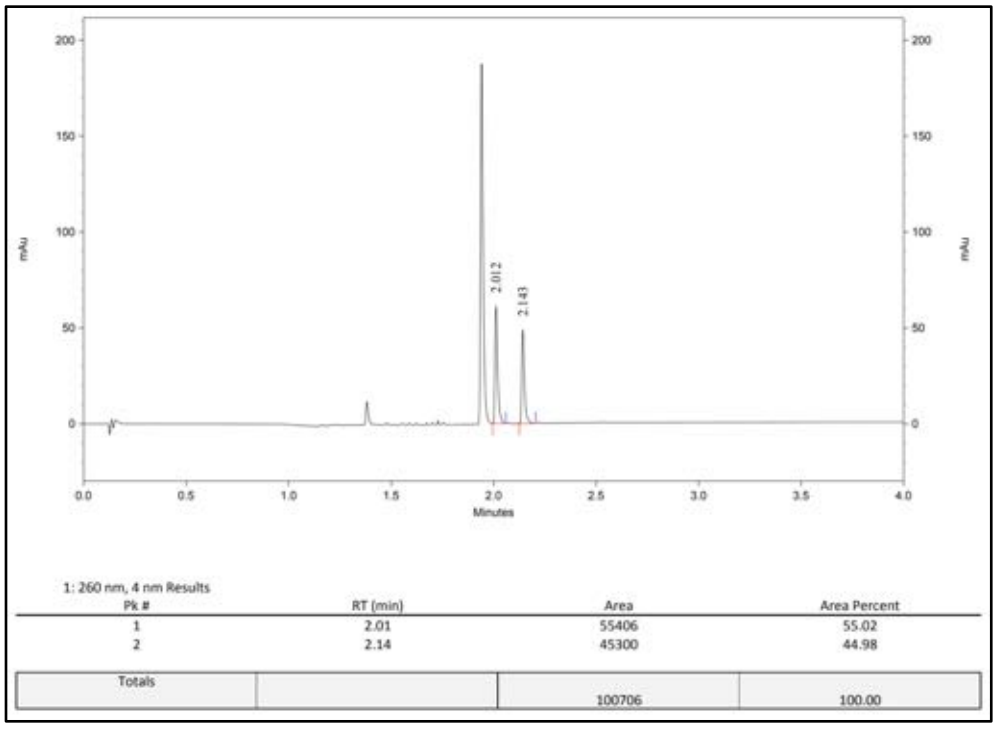
Crude mixture



1: 260 nm, 4 nm Results

Pk #	RT (min)	Area	Area Percent
1	2.02	182893	100.00
Totals		182893	100.00

After addition of the (*S*)-diastereomer (retention time = 2.14 min)



1: 260 nm, 4 nm Results

Pk #	RT (min)	Area	Area Percent
1	2.01	55406	55.02
2	2.14	45300	44.98
Totals		100706	100.00

Pictoral Guide

General Procedure 5 – Coupling



(From Left to Right): Loaded compound, nucleoside and DBU, loaded compound added to the round bottom flask, loaded compound diluted in MeCN.

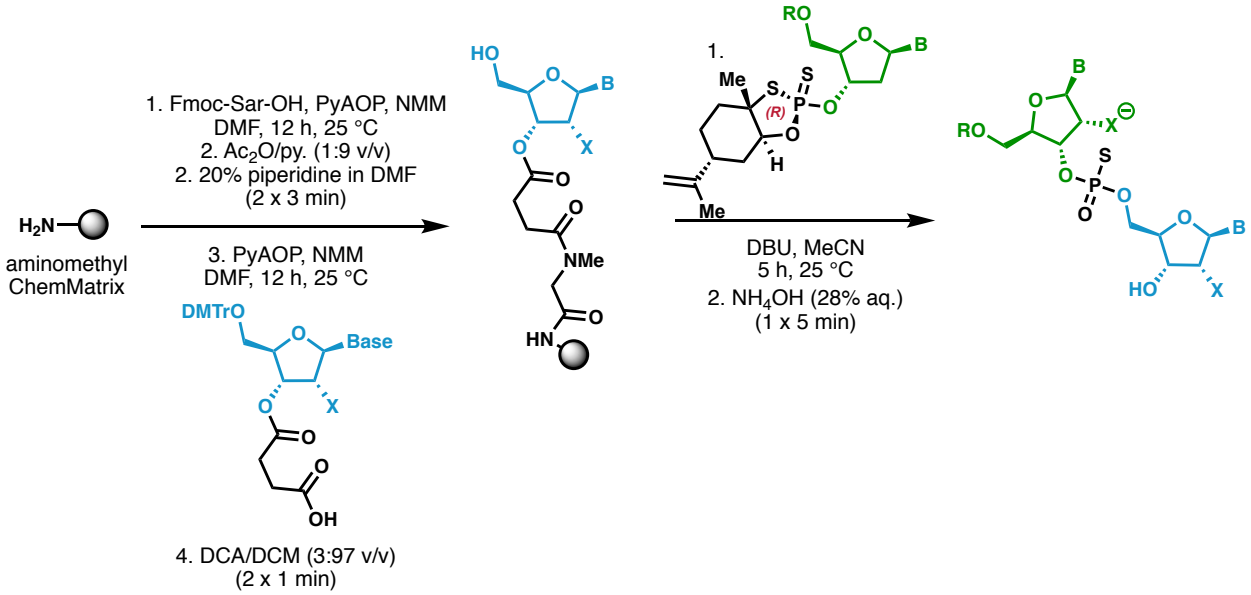


(From Left to Right): Nucleoside added to the reaction mixture, DBU added to the reaction mixture, reaction mixture stirred for 30 minutes.



(From Left to Right): Reaction mixture diluted in EtOAc and washed with $NaHCO_3$, reaction workup, desired product after column chromatography purification.

General Procedure 6 – Manual Solid-Phase Dinucleotide Synthesis



Materials

Solid-phase reaction vessels and pressure caps were purchased from Torviq.

Sarcosine–Succinate Linker

Aminomethyl ChemMatrix resin (1.0 equiv., 300 μmol, substitution = 0.56 mmol/ g) was swollen with DMF (5 x 5 mL), DCM (5 x 5 mL), then DMF (5 x 5 mL). Fmoc-Sar-OH (4.0 equiv.) and PyAOP (4.0 equiv.) were dissolved in DMF (0.1 M, final concentration relative to resin), followed by NMM (8.0 equiv.). The resulting solution was immediately added to the resin and agitated at room temperature for 12 h. The coupling solution was expelled, and the resin washed with DMF (5 x 5 mL), DCM (5 x 5 mL), then DMF (5 x 5 mL). A solution of Ac_2O/pyridine (1:9 v/v) was freshly prepared and immediately added to the resin to cap any unreacted amines; the mixture was agitated at room temperature for 3 min. The capping solution was expelled, and the resin washed with DMF (5 x 5 mL), DCM (5 x 5 mL), then DMF (5 x 5 mL).

The coupling efficiency was evaluated through treatment of the resin with a solution of 20% piperidine in DMF (3 mL, 2 x 3 min) to remove the Fmoc group. The combined deprotection solutions were diluted to 10 mL with 20% piperidine in DMF. An aliquot of this solution (25 μL) was diluted 400-fold with the same deprotection solution and the UV absorbance of the piperidine–fulvene adduct was measured ($\lambda = 301$ nm, $\varepsilon = 7800$ $M^{-1}cm^{-1}$) to quantify the amount of sarcosine coupled to the resin. The obtained value was used to calculate the reagent quantities used in the subsequent reaction.

The resin was washed with DMF (5 x 5 mL), DCM (5 x 5 mL), then DMF (5 x 5 mL). Succinylated-nucleotide (4.0 equiv., prepared according to *General Procedure 3*) and PyAOP (4.0

equiv.) were dissolved in DMF (0.1 M, relative to resin), followed by NMM (8.0 equiv.). The resulting solution was immediately added to the resin and agitated at room temperature for 5 h. The coupling solution was expelled, and the resin washed with DMF (5 x 5 mL), DCM (5 x 5 mL), then DMF (5 x 5 mL).

The coupling efficiency was evaluated according to the *General Solid-Phase Deprotection and Efficiency Evaluation* protocol. The theoretical maximum for the reported yield of the isolated dinucleotide is based on the numerical value obtained from this coupling reaction.

General Solid-Phase Loading of ψ-Compounds

The resin was washed with DCM (5 x 3 mL), DMF (5 x 3 mL), DCM (5 x 3 mL), then MeCN (5 x 2 mL). The plunger of the solid-phase vessel was carefully removed and the loaded ψ-compound (20 equiv.) was added dry to the back of the syringe; the plunger was replaced and excess air was expelled. A solution of DBU (40 equiv.) in MeCN (0.05 M final concentration, relative to resin) was added to the resin and agitated at room temperature. After 3 h, the resin was washed with MeCN (5 x 2 mL), DCM (5 x 2 mL), DMF (5 x 2 mL), then DCM (5 x 2 mL).

Cleavage

The resin was washed with H_2O (5 x 2 mL) then treated with NH_4OH (28% aq., 1 x 2 mL) and agitated for 5 min at room temperature. Following expulsion of the solution into a clean scintillation vial, the resin was wash with NH_4OH (28% aq., 3 x 2 mL) and H_2O (5 x 2 mL) to ensure complete resin cleavage. The dinucleotide was used without further purification.

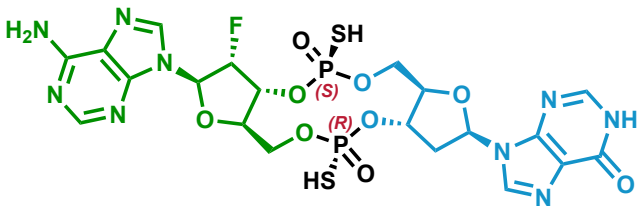
Synthesis of Cylic Dinucleotides:

Note: ^{1}H and ^{13}C NMR spectra are complicated by overlapping and split (^{19}F and ^{31}P-coupling) signals, of which broadening is observed.

HPLC analysis was conducted on a Waters Autopurification LC with a Waters XBridge C18 column (4.6x150 mm, 3.5 µm). Fractionation was triggered by a Waters QDa single quadruple mass spec in ESI+ single ion or ESI- single ions recoding modes. UV detection was monitored at 261 nm.

Solvent A: 0.1 M aqueous triethylammonium acetate
Solvent B: acetonitrile
1.5 mL/min, 25 °C.
Gradient: 5–10% B over 15 minutes

Compound (S_P, R_P)-18

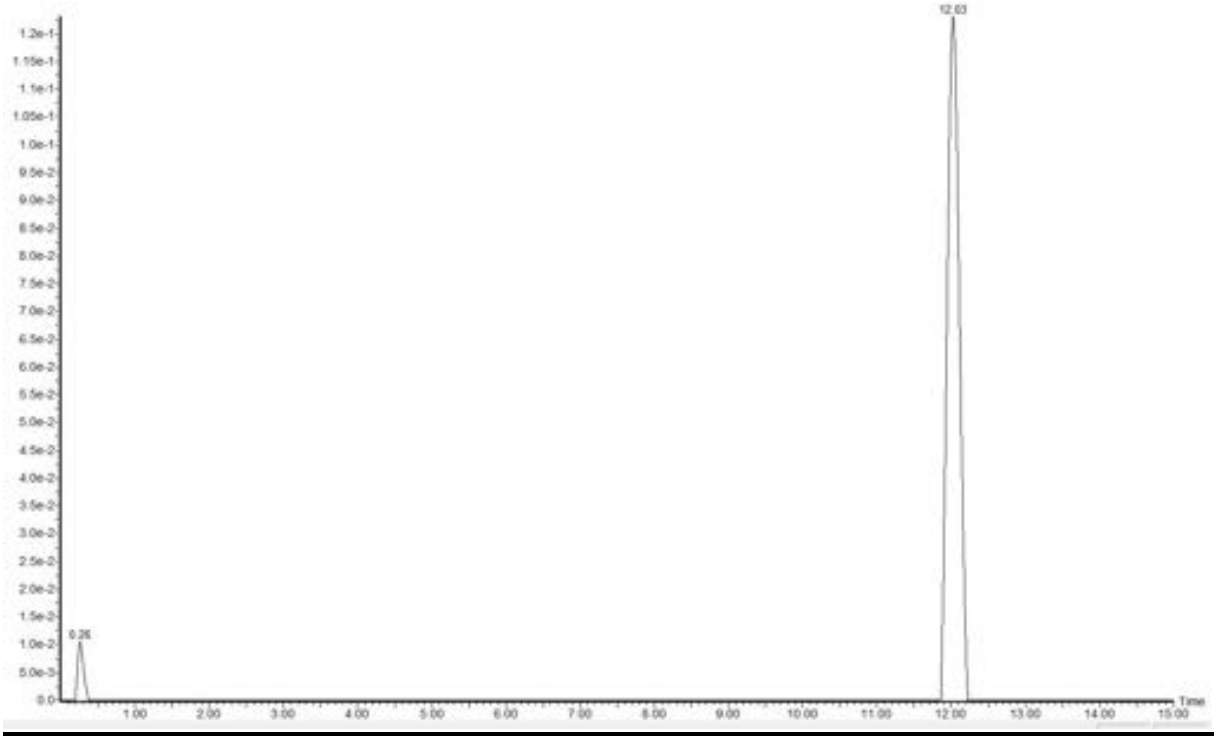


(S_P, R_P)-18

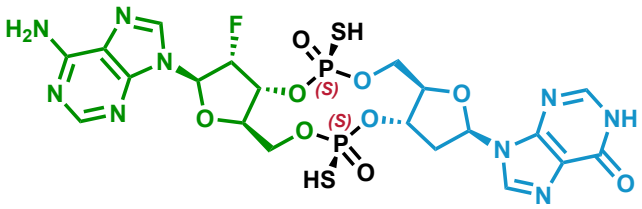
To a solution of diol **SI-(S_P)-20** (7 mg, 0.01 mmol, 1.0 equiv.) and DBU (22 µL, 0.15 mmol, 15 equiv.) in pyridine (0.2 mL, 0.05 M) was added **(–)-1** (13 mg, 0.03 mmol, 3.0 equiv.) portionwise. After stirring for 1 hour, the solvent was removed *in vacuo*. The residue was precipitated with Et_2O (1 mL). The white solid obtained was dissolved in MeCN/100 mM triethylammonium acetate (1:9 v/v, 1 mL) and purified via RP-HPLC using MeCN, 100 mM TEAA. The fractions containing product were pooled and concentrated to afford **(S_P, R_P)-18** as an amorphous solid (3.2 mg, 36%, > 99:1 *d.r*).

^{19}F NMR (376 MHz, D_2O): δ -202.92;
^{31}P NMR (162 MHz, MeOD): δ 56.17, 54.74;
HRMS (ESI) *m/z*: calculated for $C_{20}H_{21}FN_9O_9P_2S_2$ $[M-H]^-$ 676.0368; found 676.0371.

HPLC Trace of Compound (S_P, R_P)-18



Compound (S_P, S_P)-18

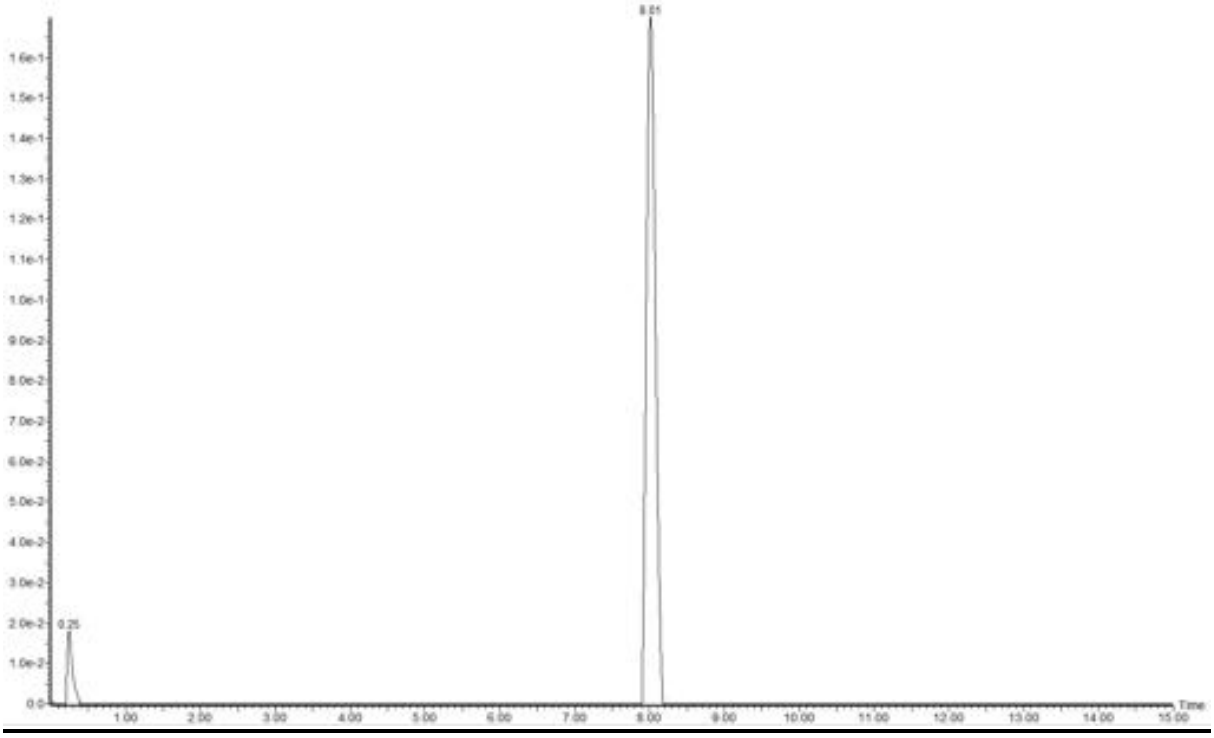


(S_P, S_P)-18

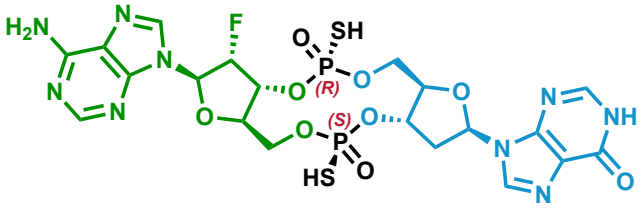
To a solution of diol **SI-(S_P)-20** (7 mg, 0.01 mmol, 1.0 equiv.) and DBU (22 µL, 0.15 mmol, 15 equiv.) in pyridine (0.2 mL, 0.05 M) was added **(–)-1** (13 mg, 0.03 mmol, 3.0 equiv.) portion-wise. After stirring for 1 hour, the solvent was removed *in vacuo*. The residue was precipitated with Et_2O (1 mL). The white solid obtained was dissolved in MeCN/100 mM triethylammonium acetate (1:9 v/v, 1 mL) and purified via RP-HPLC using MeCN, 100 mM TEAA. The fractions containing product were pooled and concentrated to afford **(S_P, R_P)-18** as an amorphous solid (4.3 mg, 49%, > 99:1 *d.r*).

^{19}F NMR (376 MHz, D_2O): δ -203.27;
^{31}P NMR (162 MHz, MeOD): δ 58.69, 58.26;
HRMS (ESI) *m/z*: calculated for $C_{20}H_{21}FN_9O_9P_2S_2$ $[M-H]^-$ 676.0368; found 676.0369.

HPLC trace of Compound (S_P, S_P)-18



Compound (R_P, S_P)-18

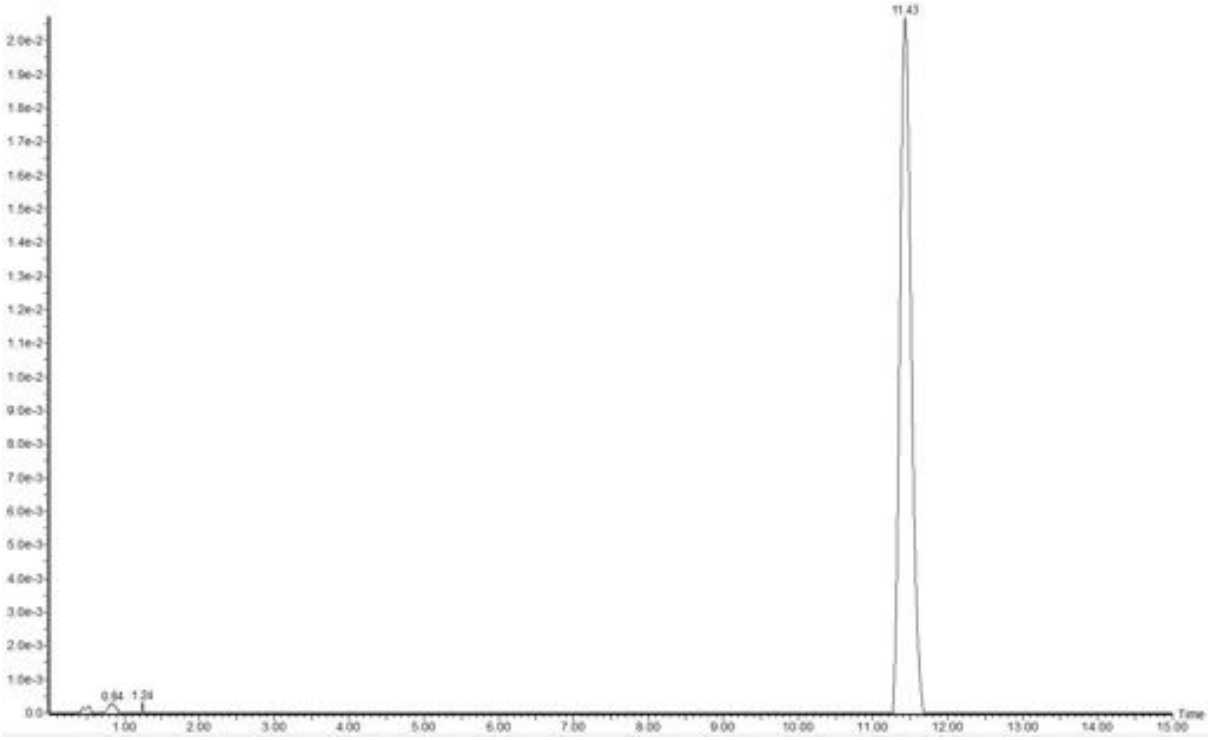


(R_P, S_P)-18

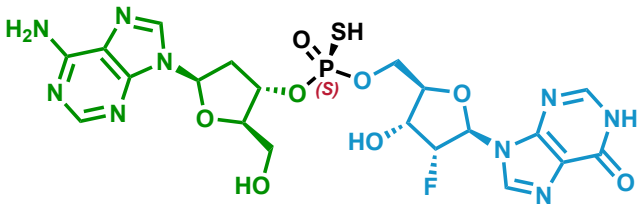
To a solution of diol **SI-(R_P)-20** (7 mg, 0.01 mmol, 1.0 equiv.) and DBU (22 µL, 0.15 mmol, 15 equiv.) in pyridine (0.2 mL, 0.05 M) was added **(–)-1** (13 mg, 0.03 mmol, 3.0 equiv.) portion-wise. After stirring for 1 hour, the solvent was removed *in vacuo*. The residue was precipitated with Et_2O (1 mL). The resulting white solid was dissolved in MeCN/100 mM triethylammonium acetate (1:9 v/v, 1 mL) and purified via RP-HPLC using MeCN, 100 mM TEAA. The fractions containing product were pooled and concentrated to afford **(S_P, R_P)-18** as an amorphous solid (2.4 mg, 27%, > 99:1 *d.r*).

^{19}F NMR (376 MHz, D_2O): δ -203.18;
^{31}P NMR (162 MHz, MeOD): δ 58.10, 57.71;
HRMS (ESI) *m/z*: calculated for $C_{20}H_{21}FN_9O_9P_2S_2$ $[M-H]^-$ 676.0368; found 676.0374.

HPLC trace of Compound (R_P, S_P)-18



Compound SI-(S_P)-19

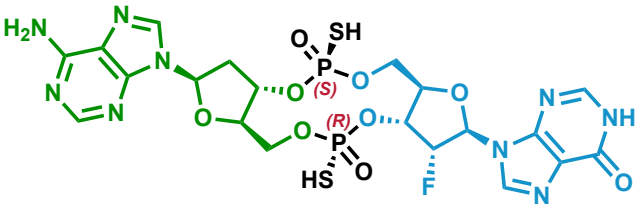


SI-(S_P)-19

Compound **SI-(S_P)-19** was prepared according to *General Procedure 6* with succinate **SI-11** (0.170 mmol), then coupled to **SI-(R_P)-17**. After concentration, **SI-(S_P)-19** was isolated as the ammonium salt. HPLC analysis determined the crude dinucleotide to be >95% purity; **SI-(S_P)-19** was used without further purification.

^{19}F NMR (376 MHz, Deuterium Oxide): δ -202.81
^{31}P NMR (162 MHz, Deuterium Oxide): δ 55.53;
HRMS (ESI) *m/z*: calculated for $C_{20}H_{23}FN_9O_8PS$ $[M+H]^+$ 600.1185; found 600.1180.

Compound (S_P, R_P)-19

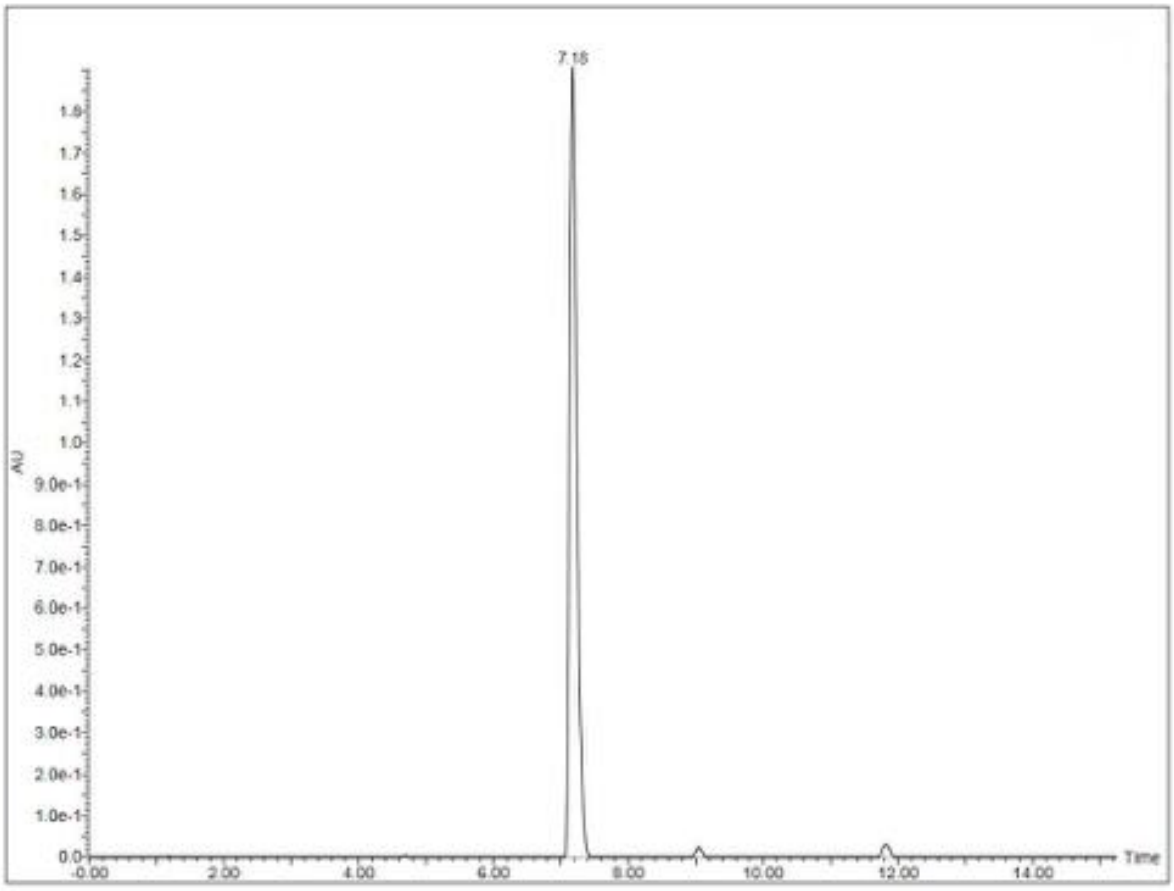


(S_P, R_P)-19

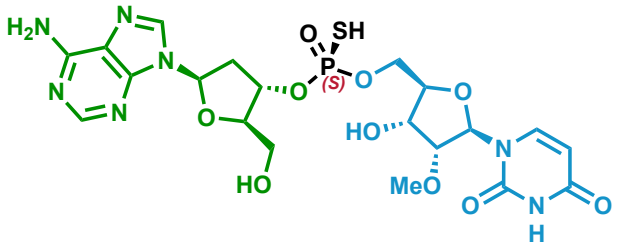
To a solution of **SI-(S_P)-19** (62 mg, 0.105 mmol, 1.0 equiv.) and DBU (0.23 mL, 1.51 mmol, 15 equiv.) in DMF (9 mL) was added a solution of **(–)-1** (60 mg, 0.154 mmol, 1.5 equiv.) in DMF (1 mL, final concentration 0.01 M) dropwise over 5 minutes. After stirring for 15 minutes, the solvent was removed *in vacuo*. The residue obtained was co-evaported with toluene (3 x 10 mL) and precipitated with Et_2O (5 mL). The white solid obtained was dissolved in MeCN/100 mM triethylammonium acetate (1:9 v/v, 2 mL) and purified via RP-HPLC using MeCN, 100 mM TEAA. The fractions containing product were pooled and concentrated to afford **(S_P, R_P)-19** as an amorphous solid, mixed triethylammonium and DBU salt (22.1 mg, .025 mmol, 24%).

^{1}H NMR (600 MHz, Deuterium Oxide): δ 8.56 (s, 1H), 8.53 (s, 1H), 8.22 (s, 1H), 8.16 (d, J = 1.2 Hz, 1H), 6.55 – 6.44 (m, 2H), 5.84 (dd, J = 51.3, 4.1 Hz, 1H), 5.33 – 5.18 (m, 1H), 5.10 (dq, J = 13.7, 7.1 Hz, 1H), 4.57 (d, J = 9.1 Hz, 1H), 4.47 – 4.33 (m, 2H), 4.25 (dt, J = 11.5, 4.3 Hz, 1H), 4.19 – 4.02 (m, 2H), 3.50 – 3.42 (m, 1H), 2.85 (dt, J = 14.2, 7.2 Hz, 1H);
^{19}F NMR (376 MHz, Methanol): δ -202.37;
^{31}P NMR (162 MHz, Methanol): δ 58.92, 57.94.
HRMS (ESI-TOF, m/z): Calcd for $C_{20}H_{22}FN_9O_9P_2S_2$ $[M + H]^+$ 678.0514; found 678.0515.
Retention Time: 7.18 min

HPLC Trace of Compound (S$_P$, R$_P$)-19



Compound (S_P)-20

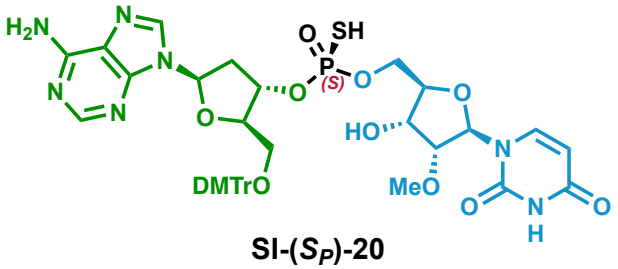


(S_P)-20

Compound **(S_P)-20** was prepared according to *General Procedure 6* with succinate **SI-12** (0.059 mmol), then coupled **SI-(R_P)-17**. After concentration, **(S_P)-20** was isolated as the ammonium salt. HPLC analysis determined the crude dinucleotide to be >95% purity; **(S_P)-20** was used without further purification.

^{31}P NMR (162 MHz, MeOD): δ57.86;
HRMS (ESI) *m/z*: calculated for $C_{20}H_{26}N_7O_{10}PS$ $[M+H]^+$ 588.1272; found 588.1274.

Compound SI-(S_P)-20

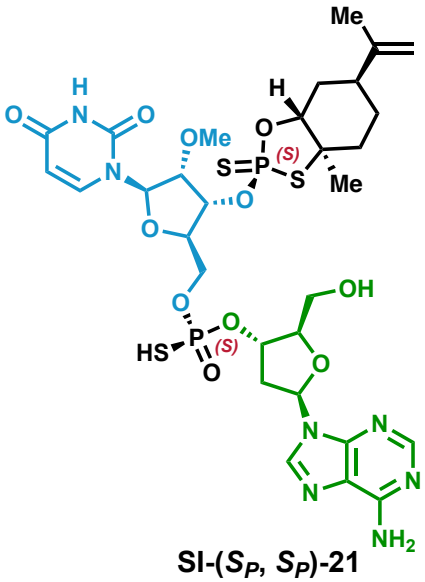


SI-(S_P)-20

Compound **SI-(S_P)-20** was prepared according to *General Procedure 6* with succinate **SI-12** (0.059 mmol), then coupled to **SI-(R_P)-18**. After concentration, **SI-(S_P)-20** was isolated as the ammonium salt. HPLC analysis determined the crude dinucleotide to be >95% purity; **SI-(S_P)-20** was used without further purification.

^{31}P NMR (162 MHz, MeOD): δ 57.90
HRMS (ESI) *m/z*: calculated for $C_{41}H_{44}N_7O_{12}PS$ $[M\text{-}DMTr+H]^+$ 588.1277; found 588.1274.

Compound SI-(S_P, S_P)-21

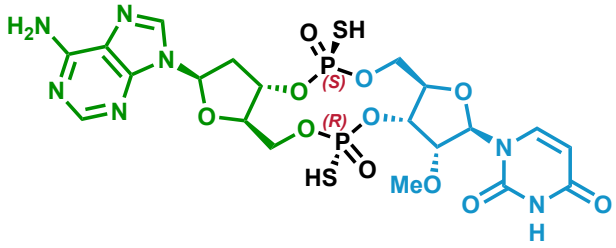


SI-(S_P, S_P)-21

Crude **SI-(S_P)-20** (0.059 mmol, *theoretical*) and **(–)-1** (112 mg, 0.250 mmol, 4.25 equiv.) was dissolved in MeCN (1.6 mL). DBU (0.38 mL, 0.250 mmol, 4.25 equiv.) was added and the mixture was stirred for 15 minutes. DCA (0.8 mL, 9.75 mmol, 165 equiv.) was then added. The solution turned bright orange and was allowed to stir for 5 minutes before being quenched (MeCN/1.0 M triethylammonium acetate, 8:2, 1 mL). The crude reaction was directly purified via RP-HPLC (MeCN, 100 mM TEAA). **SI-(S_P, S_P)-21** was isolated as the triethylammonium salt (34.7 mg, 0.037 mmol, 63% over 3 steps) and was used directly in the next reaction.

HRMS (ESI) *m/z*: calculated for $C_{30}H_{41}N_7O_{11}P2S_3$ $[M+H]^+$ 834.1574; found 834.1577.

Compound (S_P, R_P)-21



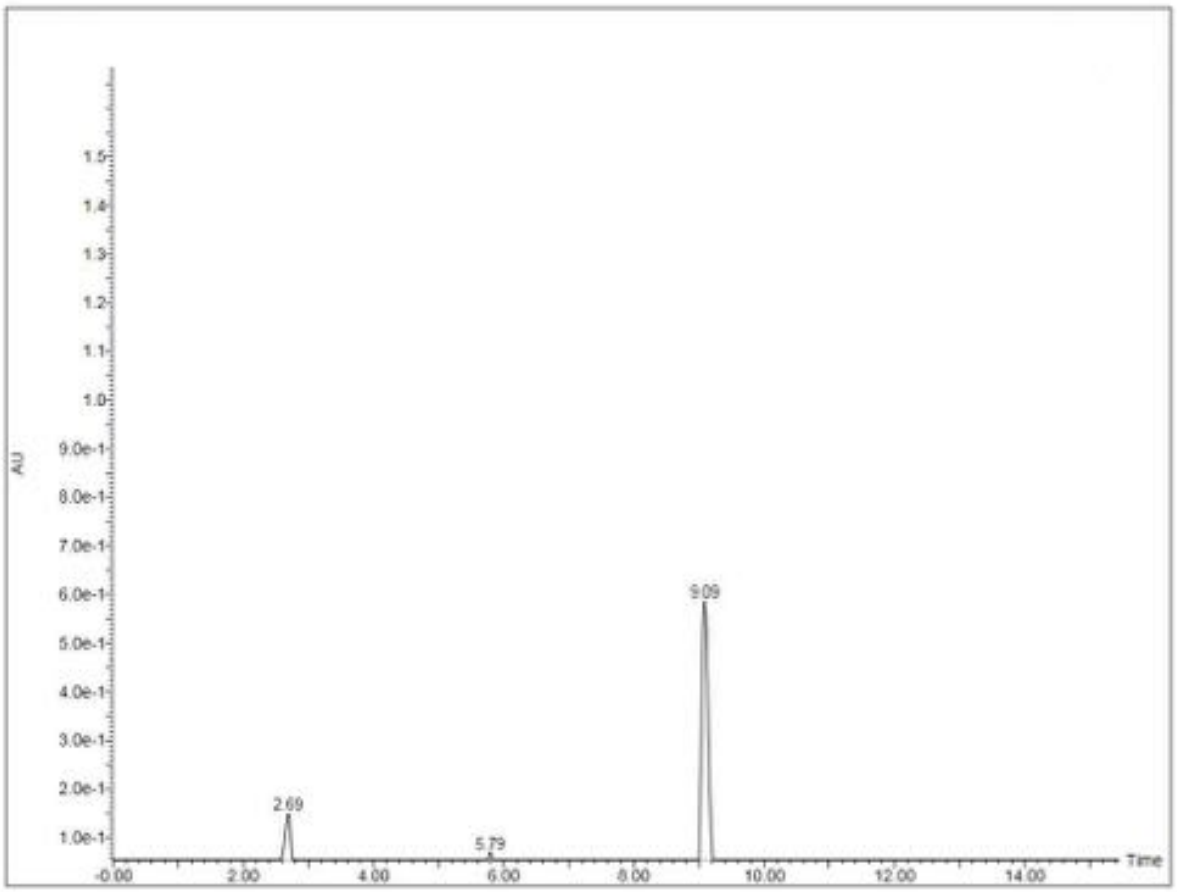
(S_P, R_P)-21

Concerted: To a solution of diol **(S_P)-20** (27 mg, 1.0 equiv.) and DBU (89 µL, 15 equiv.) in DMF (3.5 mL) was added a solution of **(–)-1** (26 mg, 1.5 equiv.) in DMF (0.5 mL, final concentration 0.01 M) dropwise over 5 minutes. After stirring for 15 minutes, the solvent was removed *in vacuo*. The residue was co-evaported with toluene (3 x 10 mL) and precipitated with Et_2O (5 mL). The white solid obtained was dissolved in MeCN/100 mM triethylammonium acetate (1:9 v/v, 1 mL) and purified via RP-HPLC using MeCN, 100 mM TEAA. The fractions containing product were pooled and concentrated to afford **(S_P, R_P)-21** as an amorphous solid (12.3 mg, 44%, 3:1 *d.r*).

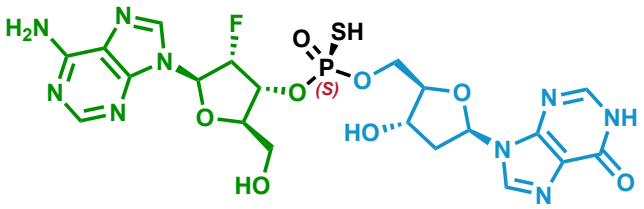
Stepwise: To a solution of **SI-(S_P)-22** (26 mg, 1.0 equiv.) in DMF (2.8 mL, 0.01 M) was added DBU (13µL, 3.0 equiv.). After stirring for 15 minutes, the solvent was removed *in vacuo*. The residue was co-evaported with toluene (3 x 5 mL) and precipitated with Et_2O (5 mL). The white solid obtained was dissolved in MeCN/100 mM triethylammonium acetate (1:9 v/v, 1 mL) and purified via RP-HPLC using MeCN, 100 mM TEAA. The fractions containing product were pooled and concentrated to afford **(S_P, R_P)-21** (5.0 mg, 14%) as the (bis)triethylammonium salt.

^{19}F NMR (376 MHz, Methanol): δ -202.37;
^{31}P NMR (162 MHz, MeOD): δ 58.10, 56.42;
HRMS (ESI) *m/z*: calculated for $C_{20}H_{25}N_7O_{11}P_2S_2$ $[M+H]^+$ 666.0601; found 666.0601.
Retention Time: 9.09 min

HPLC Trace of Compound (S$_P$, R$_P$)-21



Compound SI-(S_P)-22

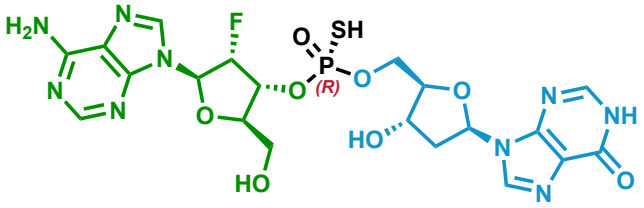


SI-(S_P)-22

(*S*)-*O*-((2*R*,3*S*,5*R*)-5-(6-amino-9*H*-purin-9-yl)-2-(((*tert*-butyldimethylsilyl)oxy)methyl)tetrahydrofuran-3-yl)*O*-(((2*R*,3*S*,5*R*)-3-((*tert*-butyldimethylsilyl)oxy)-5-(6-oxo-1*H*-purin-9(6*H*)-yl)tetrahydrofuran-2-yl)methyl) *O*-hydrogen phosphorothioate was prepared according to G*eneral Procedure 5* via the coupling of **SI-(R_P)-13** with **SI-10 (**417 mg, 0.51 mmol, 1 equiv.), and was diluted in MeCN (5.1 mL, 0.1 M). 3 HF•Et_3N (249 mg, 249 uL, 1.53 mmol, 3 equiv.) was slowly added and the reaction mixture was allowed to stir at room temperature for 18 hours. The solvent was then removed *in vacuo*, then the reaction was diluted with water (5 mL) and extracted with Et_2O (2x 5 mL). The aqueous solution was then concentrated under a stream of air. The crude desired product **SI-(S_P)-22** was obtained as a colorless gel (293 mg, 0.42 mmol, 82%) and was used in the cyclization step without further purification.

^{19}F NMR (376 MHz, D_2O): δ -202.89;
^{31}P NMR (162 MHz, MeOD): δ 54.06;
HRMS (ESI) *m/z*: calculated for $C_{20}H_{22}FN_9O_9PS$ $[M-H]^-$ 598.1039; found 598.1042.

Compound SI-(R_P)-22



SI-(R_P)-22

(*R*)-*O*-((2*R*,3*S*,5*R*)-5-(6-amino-9*H*-purin-9-yl)-2-(((*tert*-butyldimethylsilyl)oxy)methyl)tetrahydrofuran-3-yl) *O*-(((2*R*,3*S*,5*R*)-3-((*tert*-butyldimethylsilyl)oxy)-5-(6-oxo-1*H*-purin-9(6*H*)-yl)-tetrahydrofuran-2-yl)methyl) *O*-hydrogen phosphorothioate was prepared according to G*eneral Procedure 5* via coupling of **SI-(S_P)-13** with **SI-10** (193 mg, 0.21 mmol, 1 equiv.) and was diluted in MeCN (2.1 mL, 0.1 M). 3 HF•Et_3N (102 mg, 102 uL, 1.53 mmol, 3 equiv.) was slowly added and the reaction mixture was allowed to stir at room temperature for 18 hours. The solvent was removed *in vacuo* and the reaction was diluted with water (3 mL) and extracted with Et_2O (2 x 3 mL). The aqueous solution was then concentrated under a stream of air. The crude desired product **SI-(R_P)-22** was obtained as a colorless gel (144 mg, 0.42 mmol, 98%) and was used in the cyclization step without further purification.

^{19}F NMR (376 MHz, D_2O): δ -204.27;
^{31}P NMR (162 MHz, MeOD): δ 55.86;
HRMS (ESI) *m/z*: calculated for $C_{20}H_{22}FN_9O_9PS$ $[M-H]^-$ 598.1039; found 598.1038.

Pictoral Guide

CDN Synthesis – TBS Deprotection



(From Left to Right): Coupled compound, coupled compound diluted in MeCN, $HF_3.Et_3N$.



(From Left to Right): Dropwise addition of $HF_3.Et_3N$, reaction mixture after being stirred for 3h, desired diol product after preparative HPLC purification.

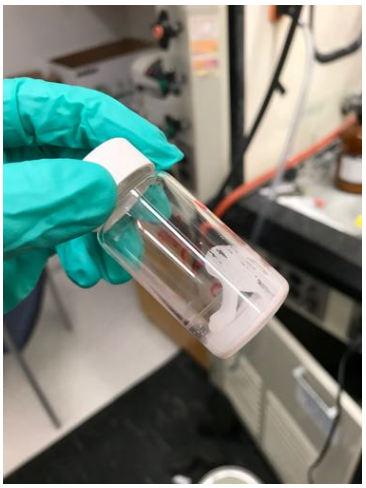
CDN Macrocyclization

(From Left to Right): Diol compound, PSI reagent and DBU, diol compound added to the round bottom flask, diol compound diluted in pyridine.



(From Left to Right): DBU added to the reaction mixture, PSI reagent added portion-wise to the reaction mixture, reaction mixture stirred for 1 hour.



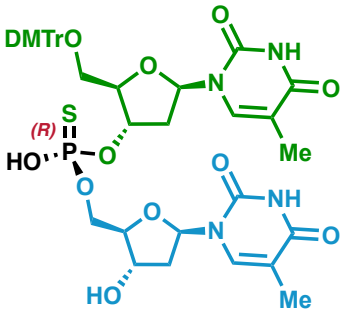


(From Left to Right): Reaction mixture diluted in EtOAc and washed with $NaHCO_3$, reaction workup, desired product after preparative HPLC purification

Preparative HPLC purification system


Synthesis of (R_P) and (S_P)-dT Standards

Compound SI-(R_P)-23



SI-(R_P)-23

1-((2R,4S,5R)-5-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-4-(((1S,3S,3aR)-3-phenyltetrahydro-1H,3H-pyrrolo[1,2-c][1,3,2]oxazaphosphol-1-yl)oxy)tetrahydrofuran-2-yl)-5-methylpyrimidine-2,4(1H,3H)-dione (*s-dT phosphoramidite*) was synthesized in a manner analogous to the procedure outlined by Wada *et al.* (*13*). Synthesis was carried out on an Äkta Oligopilot 150 oligonucleotide synthesizer on a 50 mmol scale using 5'-DMT-T-lcaa-CPG 500A resin (Chem-Impex, 00380). Each synthesis cycle used the following parameters:

Step Operation Reagents and Solvents Parameters

Step	Operation	Reagent/Solvent	Parameters
1	Detritylation	3% Dichloroacetic acid in toluene	300 cm/h flow rate until UV signal at 350 nm is <200 mA
2	Coupling	0.1 M *s-dT Phosphoramdite* in isobytyronitrile (10 eq, 40%), 1.0 M 4,5-dicyanoimidazole, 0.1M 1-methylimidazole in acetonitrile (60%)	400 cm/h flow rate with 20 minute recycle
3	Coupling	0.1 M *s-dT Phosphoramdite* in isobytyronitrile (10 eq, 40%), 1.0 M 4,5-dicyanoimidazole, 0.1M 1-methylimidazole in acetonitrile (60%)	400 cm/h flow rate with 20 minute recycle
4	Coupling	0.1 M *s-dT Phosphoramdite* in isobytyronitrile (10 eq, 40%), 1.0 M 4,5-dicyanoimidazole, 0.1M 1-methylimidazole in acetonitrile (60%)	400 cm/h flow rate with 20 minute recycle
5	Oxidation	0.1M xanthane in 3:2 pyridine - acetonitrile	3 minute flow, 2 CV, then 5 CV contact time

An acetonitrile wash of 4-6 CV was completed following each step of the coupling cycle. Once the synthesis was complete, the column was removed from the synthesizer. Air was pulled though the column for 30 minutes. The resin was then transferred to a 40 mL scintillation vial. Saturated aqueous ammonium hydroxide (15 mL) was added to the vial which was then tightly sealed. Mixture was warmed to 55 °C and held for 48 hours. The mixture was cooled and then filtered.

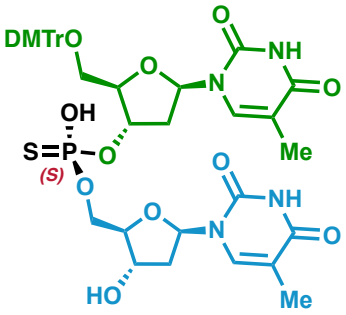
The filter cake was washed three times with 50% ethanol–water. The filtrate was concentrated *in vacuo*. The resulting residue was dissolved in 0.1 M aqueous sodium hydroxide (15 mL). The mixture was washed three times with dichloromethane. The aqueous phase was made acidic with 1 M aqueous citric acid (5 mL) and extracted three times with ethyl acetate. The combined organic layers were washed with brine then dried over $MgSO_4$. The mixture was filtered and the filtrate was treated with triethylamine (0.5 mL) before being concentrated to dryness. O-((2R,3S,5R)-2-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-5-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)tetrahydrofuran-3-yl) O-(((2R,3S,5R)-3-hydroxy-5-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)tetrahydrofuran-2-yl)methyl) S-hydrogen (R)-phosphorothioate, triethylammonium salt (24.2 mg, 0.021 mmol, 42% yield) was obtained as a clear colorless residue.

^{1}H NMR (500 MHz, **Chloroform-*d***) δ 7.66 (br d, J=0.9 Hz, 1H), 7.61 (br d, J=0.9 Hz, 1H), 7.41 (br d, J=7.5 Hz, 3H), 7.30 (br d, J=8.5 Hz, 4H), 7.24 (br d, J=7.3 Hz, 1H), 7.18 (d, J=8.9 Hz, 2H), 6.84 (d, J=8.9 Hz, 4H), 6.40 (dd, J=8.9, 5.3 Hz, 1H), 6.32 (t, J=6.4 Hz, 1H), 5.36 - 5.32 (m, 1H), 4.63 - 4.55 (m, 1H), 4.37 (br d, J=1.1 Hz, 1H), 4.27 - 4.15 (m, 2H), 4.05 (br d, J=3.5 Hz, 1H), 3.79 (s, 5H), 3.54 - 3.37 (m, 2H), 2.65 - 2.57 (m, 1H), 2.42 - 2.30 (m, 2H), 2.27 - 2.20 (m, 1H), 2.06 (s, 3H), 1.96 (d, J=0.8 Hz, 3H).

^{31}P NMR (202 MHz, **Chloroform-*d***) δ 57.8 (q, J=10.0 Hz, 1P).

LC/MS (Phenomonex Luna C18 2 x 30 mm 3µ; 5% CH3CN-H2O 10µM ammonium acetate to 95% CH_3CN-H_2O 10µM ammonium acetate over 2 min at 1 mL/min, 40 °C) (M-H)-= 863.3 (rf 1.4 min).

Compound SI-(S_P)-23



SI-(S_P)-23

1-((2R,4S,5R)-5-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-4-(((1R,3R,3aS)-3-phenyltetrahydro-1H,3H-pyrrolo[1,2-c][1,3,2]oxazaphosphol-1-yl)oxy)tetrahydrofuran-2-yl)-5-methylpyrimidine-2,4(1H,3H)-dione (*r-dT phosphoramidite*) was synthesized in a manner analogous to the procedure outlined by Wada *et al*. (*13*). Synthesis was carried out on an Äkta Oligopilot 150 oligonucleotide synthesizer on a 50 mmole scale using 5'-DMT-T-lcaa-CPG 500A resin (Chem-Impex, 00380,). Each synthesis cycle used the following parameters:

Step Operation Reagents and Solvents Parameters

Step	Operation	Reagent/Solvent	Parameters

1	Detritylation	3% Dichloroacetic acid in toluene	300 cm/h flow rate until UV signal at 350 nm is <200 mA
2	Coupling	0.1 M *r-dT Phosphoramdite* in isobytyronitrile (10 eq, 40%), 1.0 M 4,5-dicyanoimidazole, 0.1M 1-methylimidazole in acetonitrile (60%)	400 cm/h flow rate with 20 minute recycle
3	Coupling	0.1 M *r-dT Phosphoramdite* in isobytyronitrile (10 eq, 40%), 1.0 M 4,5-dicyanoimidazole, 0.1M 1-methylimidazole in acetonitrile (60%)	400 cm/h flow rate with 20 minute recycle
4	Coupling	0.1 M *r-dT Phosphoramdite* in isobytyronitrile (10 eq, 40%), 1.0 M 4,5-dicyanoimidazole, 0.1M 1-methylimidazole in acetonitrile (60%)	400 cm/h flow rate with 20 minute recycle
5	Oxidation	0.1M xanthane in 3:2 pyridine - acetonitrile	3 minute flow, 2 CV, then 5 CV contact time

An acetonitrile wash of 4-6 CV was completed following each step of the coupling cycle. Once the synthesis was complete, the column was removed from the synthesizer. Air was pulled though the column for 30 minutes. The resin was then transferred to a 40 mL scintillation vial. Saturated aqueous ammonium hydroxide (15 mL) was added to the vial which was then tightly sealed. Mixture was warmed to 55 °C and held for 44 hours. The mixture was cooled and then filtered. The filter cake was washed three times with 50% ethanol–water. Filtrate was concentrated *in vacuo*. The resulting residue was dissolved in 0.1 M aqueous sodium hydroxide (15 mL). The mixture was washed three times with dichloromethane. The aqueous phase was made acidic with 1 M aqueous citric acid (5 mL). Material was extracted three times with ethyl acetate. Combined organics were washed with brine. Organics were dried $MgSO_4$. Mixture was filtered and the filtrate was treated with triethylamine (1.0 mL) before being concentrated to dryness. **SI-(S_P)-23** was isolated as the triethylammonium salt (23 mg, 0.019 mmol, 38 % yield) as a clear colorless residue.

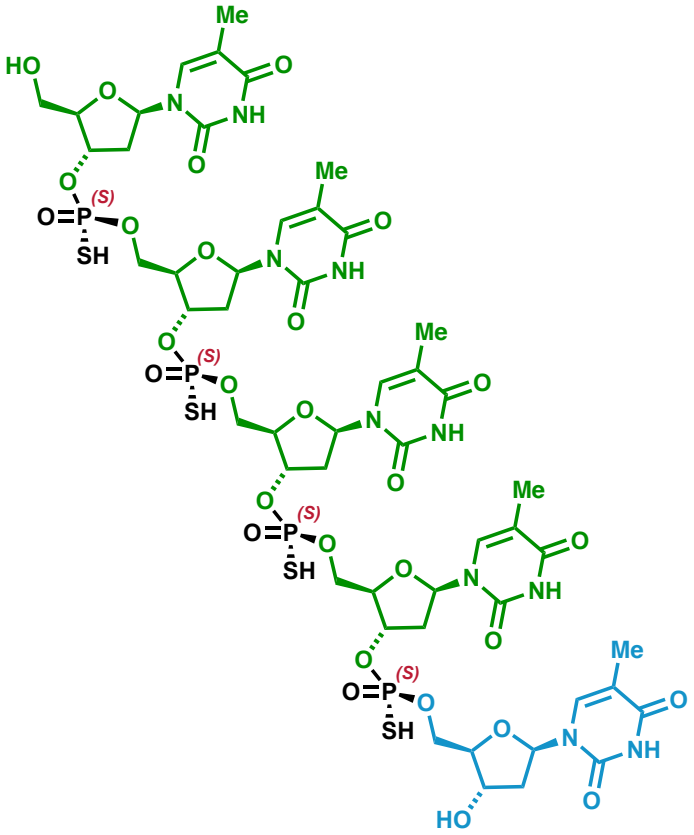
^{1}H NMR (500 MHz, MeOD) δ 7.81 (d, J=1.1 Hz, 1H), 7.69 (d, J=1.1 Hz, 1H), 7.46 (br d, J=7.5 Hz, 2H), 7.37 - 7.30 (m, 6H), 7.26 - 7.22 (m, 1H), 6.88 (d, J=8.9 Hz, 4H), 6.38 (dd, J=8.3, 5.7 Hz, 1H), 6.32 (dd, J=8.0, 6.0 Hz, 1H), 5.45 - 5.36 (m, 1H), 4.45 (dt, J=5.4, 2.5 Hz, 1H), 4.30 (br d, J=2.1 Hz, 1H), 4.17 - 4.09 (m, 1H), 4.03 - 3.96 (m, 2H), 3.79 (s, 7H), 3.50 (dd, J=10.5, 2.8 Hz, 1H), 3.37 (dd, J=10.5, 2.6 Hz, 1H), 3.11 - 3.04 (m, 1H), 2.64 - 2.59 (m, 1H), 2.49 (ddd, J=14.0, 8.2, 6.2 Hz, 1H), 2.26 - 2.12 (m, 2H), 1.93 (d, J=0.8 Hz, 3H), 1.35 (s, 3H).

^{31}P NMR (202 MHz, MeOD) δ 57.6 (br d, J=7.5 Hz, 1P).

LC/MS (Phenomonex Luna C18 2 x 30 mm 3µ; 5% CH3CN-H2O 10µM ammonium acetate to 95% CH3CN-H2O 10µM ammonium acetate over 2 min at 1 mL/min, 40 °C) $(M-H)^-$= 863.3 (rf 1.4 min).

Automated Solid-Phase Oligonucleotide Synthesis

Compound-(S_P, S_P, S_P, S_P)-23



(S_P, S_P, S_P, S_P, S_P)-23

Synthesis was carried out on a MerMade MM12 oligonucleotide synthesizer on a 2.5 mmol scale using dT-Q-CPG 500 oligonucleotide synthesis resin (Glen Research, 20-2030-XX). Each synthesis cycle used the following parameters:

Step Operation Reagents and Solvents Parameters

Step	Operation	Reagent/Solvent	Time
1	Detritylation	3% Dichloroacetic acid in dichloromethane (1 mL)	90 sec.
2	Detritylation	3% Dichloroacetic acid in dichloromethane (1 mL)	90 sec.
3	ACN Wash	Acetonitrile (1.5 mL)	20 sec.
4	ACN Wash	Acetonitrile (1.5 mL)	20 sec.
5	Coupling	0.1 M (R)-Ψ-dT in acetonitrile (0.2 mL, 8 equiv.), 1.0 M 1,8-diazabicyclo[5.4.0]undec-7-ene in acetonitrile (0.1 mL, 40 equiv.)	300 sec.
6	Coupling	0.1 M (R)-Ψ-dT in acetonitrile (0.2 mL, 8 equiv.), 1.0 M 1,8-diazabicyclo[5.4.0]undec-7-ene in acetonitrile (0.1 mL, 40 equiv.)	300 sec.
7	ACN Wash	Acetonitrile (1.5 mL)	20 sec.

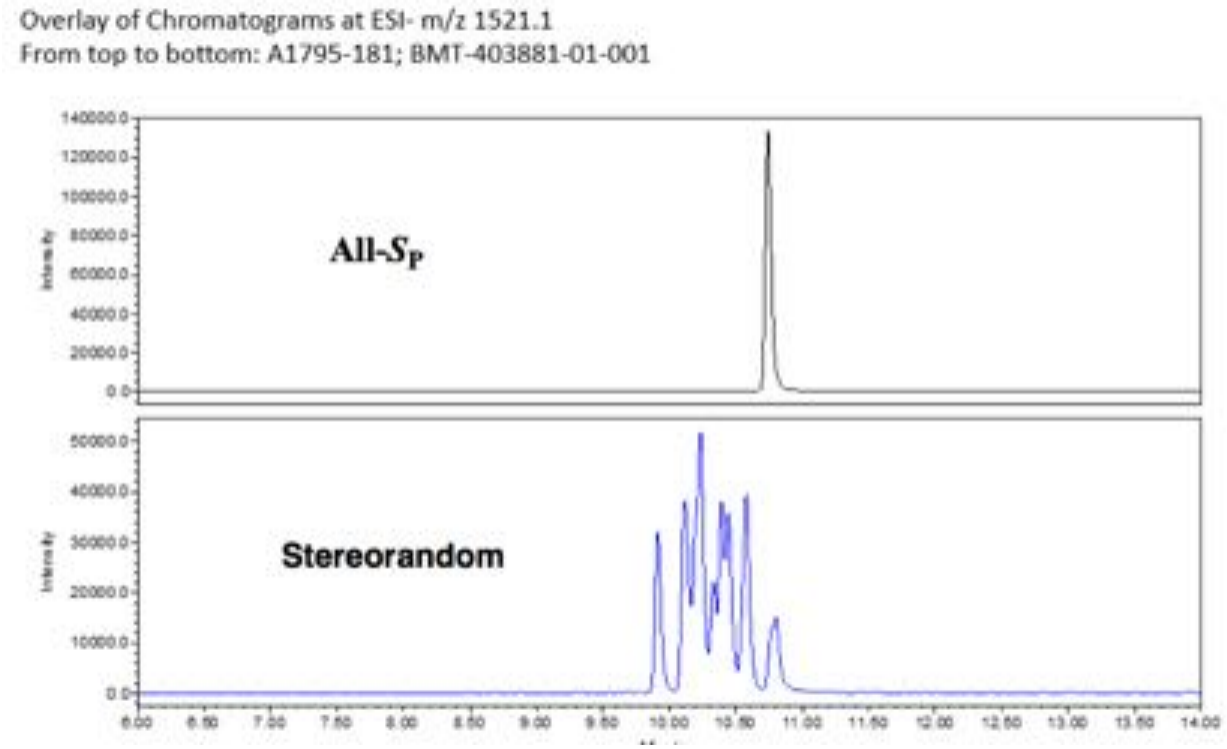
8	ACN Wash	Acetonitrile (1.5 mL)	20 sec.
9	Capping	20% acetic anhydride, 30% 2,6-lutidine, 50% acetonitrile (0.4 mL) 20% N-methylimidazole in acetonitrile (0.4 mL)	60 sec.
10	ACN Wash	Acetonitrile (1.5 mL)	20 sec.
11	ACN Wash	Acetonitrile (1.5 mL)	20 sec.

At the completion of the synthesis the column was subjected to two more detritylation steps followed by two more acetonitrile washes. The column was removed from the synthesizer. Air was pulled through the column for 20 minutes. The resin was collected in a 2 dram scintillation vial. Saturated aqueous ammonium hydroxide (2 mL) was added to the vial which was then tightly sealed. Mixture was warmed to 55 °C and held for 1.5 hours. Mixture was cooled to room temperature and then filtered. The filter cake was washed twice with 50% ethanol – water. The filtrate was concentrated *in vacuo* to afford the crude oligonucleotide (5 mg) as a white film.

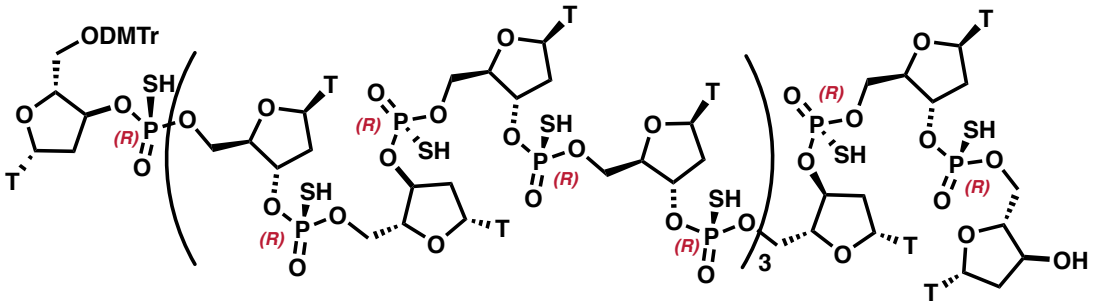
LCMS (Aquity UPLC Oligo BEH, C18, 1.7 μ, 2.1x50 mm; Solvent A: 97.5% water, 2.5% methanol, 0.2M 1,1,1,3,3,3-hexafluoro-2-propanol, 16 mmol triethylamine; Solvent B: 40% water, 60% methanol, 0.2M 1,1,1,3,3,3-hexafluoro-2-propanol, 16 mmol triethylamine; 100% A isocratic over 0.5 min then 100% A to 25% B-A over 2.25 min then 25% B-A to 100% B over 0.5 min at 1 mL/min, 65 °C) m/2- = 760.4 (rf 1.44 min, ~73% purity). The crude film was purified by preparatory HPLC (Waters XBridge, C18, 5 μ, 19x100 mm; Solvent A: 98% water, 2% methanol, 0.4M 1,1,1,3,3,3-hexafluoro-2-propanol, 16 mmol triethylamine; Solvent B: 40% water, 60% methanol, 0.4M 1,1,1,3,3,3-hexafluoro-2-propanol, 16 mmol triethylamine; run gradient 100% A to 40% B-A over 18.5 minutes at 20 mL/min). The fractions containing product were pooled and concentrated using a Biotage V10 evaporation system to afford **(*S*$_P$, *S*$_P$, *S*$_P$, *S*$_P$)-23** (1.0 mg, 26% yield).

LCMS analysis of (S_P, S_P, S_P, S_P)-23

Column: Atlantis T3 4.6 x 150 mm, 3 µm particle size, PN 186003729, Mobile Phase A: 0.01 M TEAA: Acetonitrile (98:1), Mobile Phase B: MeCN. Gradient: 0/15/20 min, 0/30/90 %B. 25 °C, 1 mL/min.



Compound-(*All-R*$_P$)-24



(*All-R*$_P$)-24

Synthesis was carried out on a MerMade MM12 oligonucleotide synthesizer on a 2.5 µmole scale using dT-Q-CPG 500 oligonucleotide synthesis resin (Glen Research, 20-2030-XX). Each synthesis cycle used the following parameters:

Step Operation Reagents and Solvents Parameters

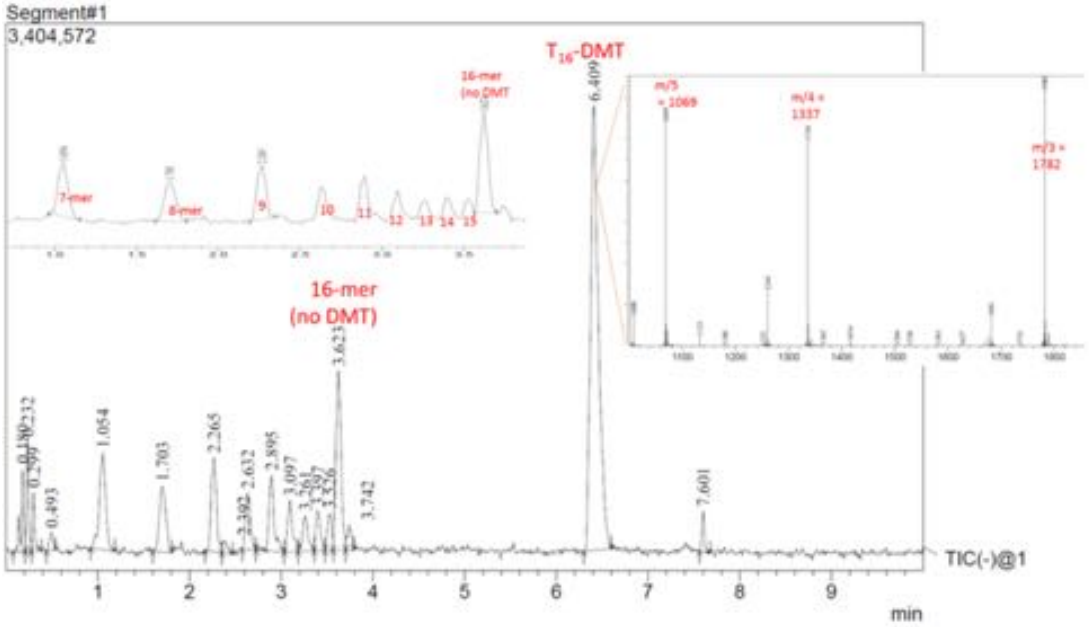
Step	Operation	Reagent/Solvent	Time
1	Detritylation	3% Dichloroacetic acid in dichloromethane (1 mL)	90 sec.
2	Detritylation	3% Dichloroacetic acid in dichloromethane (1 mL)	90 sec.
3	ACN Wash	Acetonitrile (1.5 mL)	20 sec.
4	ACN Wash	Acetonitrile (1.5 mL)	20 sec.
5	Coupling	0.1 M (S)-Ψ-dT in acetonitrile (0.2 mL, 8 equiv.), 1.0 M 1,8-diazabicyclo[5.4.0]undec-7-ene in acetonitrile (0.1 mL, 40 equiv.)	300 sec.
6	Coupling	0.1 M (S)-Ψ-dT in acetonitrile (0.2 mL, 8 equiv.), 1.0 M 1,8-diazabicyclo[5.4.0]undec-7-ene in acetonitrile (0.1 mL, 40 equiv.)	300 sec.
7	Coupling	0.1 M (S)-Ψ-dT in acetonitrile (0.2 mL, 8 equiv.), 1.0 M 1,8-diazabicyclo[5.4.0]undec-7-ene in acetonitrile (0.1 mL, 40 equiv.)	300 sec.
8	ACN Wash	Acetonitrile (1.5 mL)	20 sec.
9	ACN Wash	Acetonitrile (1.5 mL)	20 sec.
10	Capping	20% acetic anhydride, 30% 2,6-lutidine, 50% acetonitrile (0.4 mL) 20% N-methylimidazole in acetonitrile (0.4 mL)	60 sec.
11	ACN Wash	Acetonitrile (1.5 mL)	20 sec.
12	ACN Wash	Acetonitrile (1.5 mL)	20 sec.

At the completion of the synthesis the column was subjected to two more acetonitrile washes. The column was removed from the synthesizer. Air was pulled through the column for 20 minutes. The resin in the column was treated with saturated aqueous ammonium hydroxide (3x 1mL), eluting into a scintillation vial. The eluent was concentrated *in vacuo*.

LCMS (Aquity UPLC Oligo BEH, C18, 1.7 µ, 2.1x50 mm; Solvent A: 97.5% water, 2.5% methanol, 0.2M 1,1,1,3,3,3-hexafluoro-2-propanol, 16 mmol triethylamine; Solvent B: 40%

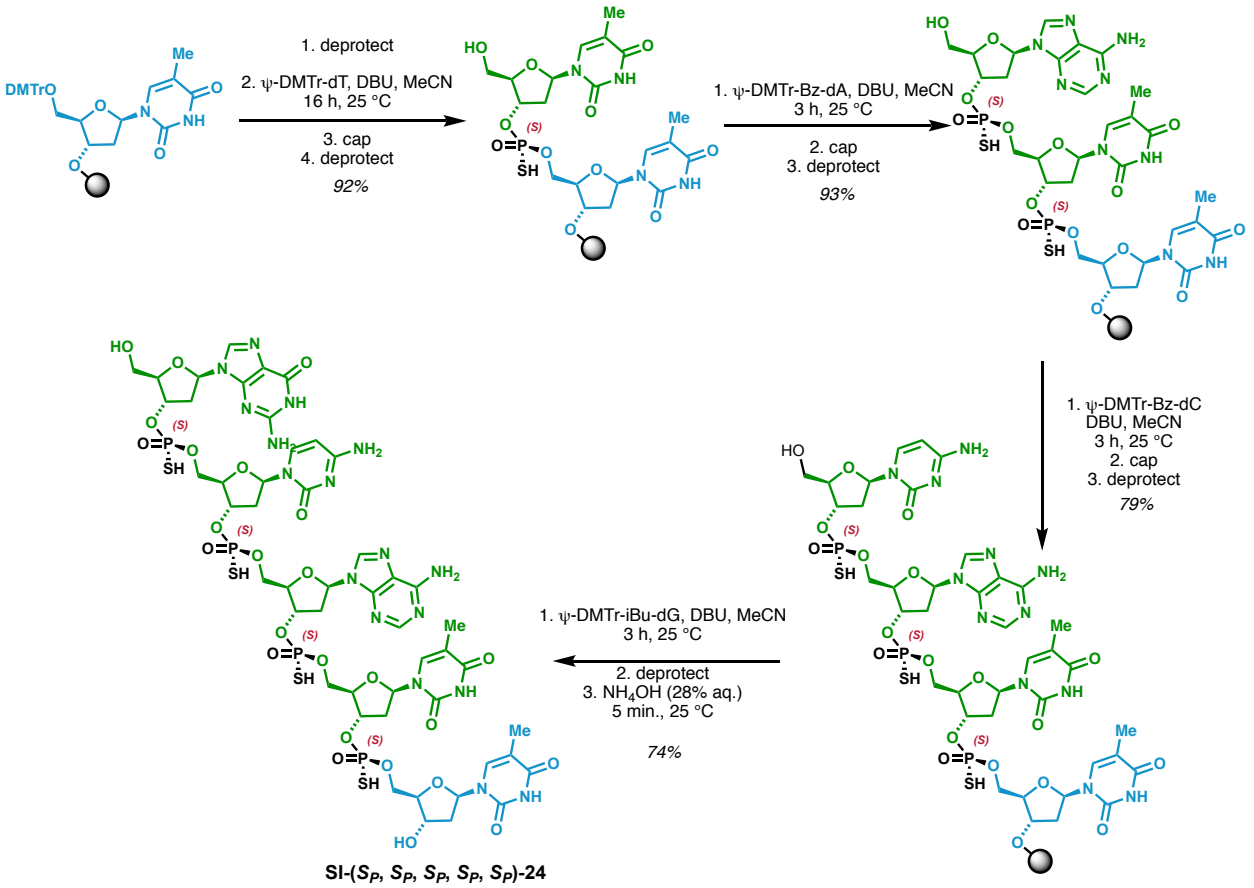
water, 60% methanol, 0.2M 1,1,1,3,3,3-hexafluoro-2-propanol, 16 mmol triethylamine; 10% B isocratic over 1min, then 10% B to 60% B over 6 min then 60% B to 100% B over 0.5 min and holding at 100%B for 1.5 min, all at 1 mL/min, 65 °C) m/3- = 1782 (rf 6.5 min, 34% purity at 262nm).

LCMS analysis of (All-R_P)-24



Peak#	Ret. Time	Area	m/z	Area%	ID
1	0.18	1088561	TIC	2.644	2-mer
2	0.232	1147090	TIC	2.786	3-mer
3	0.299	666461	TIC	1.618	4-mer
4	0.493	343066	TIC	0.833	5-mer
5	1.054	2955373	TIC	7.177	6-mer
6	1.703	2109015	TIC	5.122	7-mer
7	2.265	2431951	TIC	5.906	8-mer
8	2.392	301609	TIC	0.732	9-mer
9	2.632	1324868	TIC	3.217	10-mer
10	2.895	1956454	TIC	4.751	11-mer
11	3.097	1207687	TIC	2.933	12-mer
12	3.261	989152	TIC	2.402	13-mer
13	3.397	918543	TIC	2.231	14-mer
14	3.526	809065	TIC	1.965	15-mer
15	3.623	4452391	TIC	10.813	16-mer -DMT
16	3.742	505963	TIC	1.229	?
17	6.409	17471106	TIC	42.428	16-mer +DMT
18	7.601	499750	TIC	1.214	?
Total		41178104		100	

Manual Solid-Phase Oligonucleotide Synthesis



SI-(S_P, S_P, S_P, S_P, S_P)-24

Materials

Solid-phase reaction vessels and pressure caps were purchased from Torviq. DMTr-dT-Q-CPG was purchased from Glen Research.

General Solid-Phase Deprotection and Efficiency Evaluation

The coupling efficiency was evaluated through treatment of the resin with a solution of dichloroacetic acid (DCA)/DCM (3:97 v/v) (1 mL, 2 x 1 min.) to remove the DMTr group. The combined deprotection solutions were diluted to 10 mL with DCM/DCM (3:97 v/v). An aliquot of this solution (25 µL) was diluted 400-fold with DCM/DCM (3:97 v/v) and the UV absorbance of the DMTr cation was measured (λ = 410 nm, ε = 30,400 $M^{-1}cm^{-1}$) to quantify the amount of nucleotide coupled to the resin-bound substrate. The theoretical maximum for the reported yield of the isolated oligonucleotide is based on the numerical value obtained from the initial coupling reaction. The theoretical maximum for the reported UV yields are based on the numerical value obtained in each preceding coupling reaction.

General Solid-Phase Loading of ψ-Compounds

Deprotected dT-Q-CPG (1.0 equiv., 5 μmol, substitution = 51 μmol/100 mg) was washed with DCM (5 x 3 mL), DMF (5 x 3 mL), DCM (5 x 3 mL), then MeCN (5 x 2 mL). The plunger of the solid-phase vessel was carefully removed and the loaded ψ-compound (20 equiv.) was added dry to the back of the syringe; the plunger was replaced and excess air was expelled. A solution of DBU (40 equiv.) in MeCN (0.05 M final concentration, relative to resin) was added to the resin and agitated at room temperature. After 3 h, the resin was washed with MeCN (5 x 2 mL), DCM (5 x 2 mL), DMF (5 x 2 mL), then DCM (5 x 2 mL). Unreacted alcohol was then capped (detailed below) to minimize formation of undesired truncations.

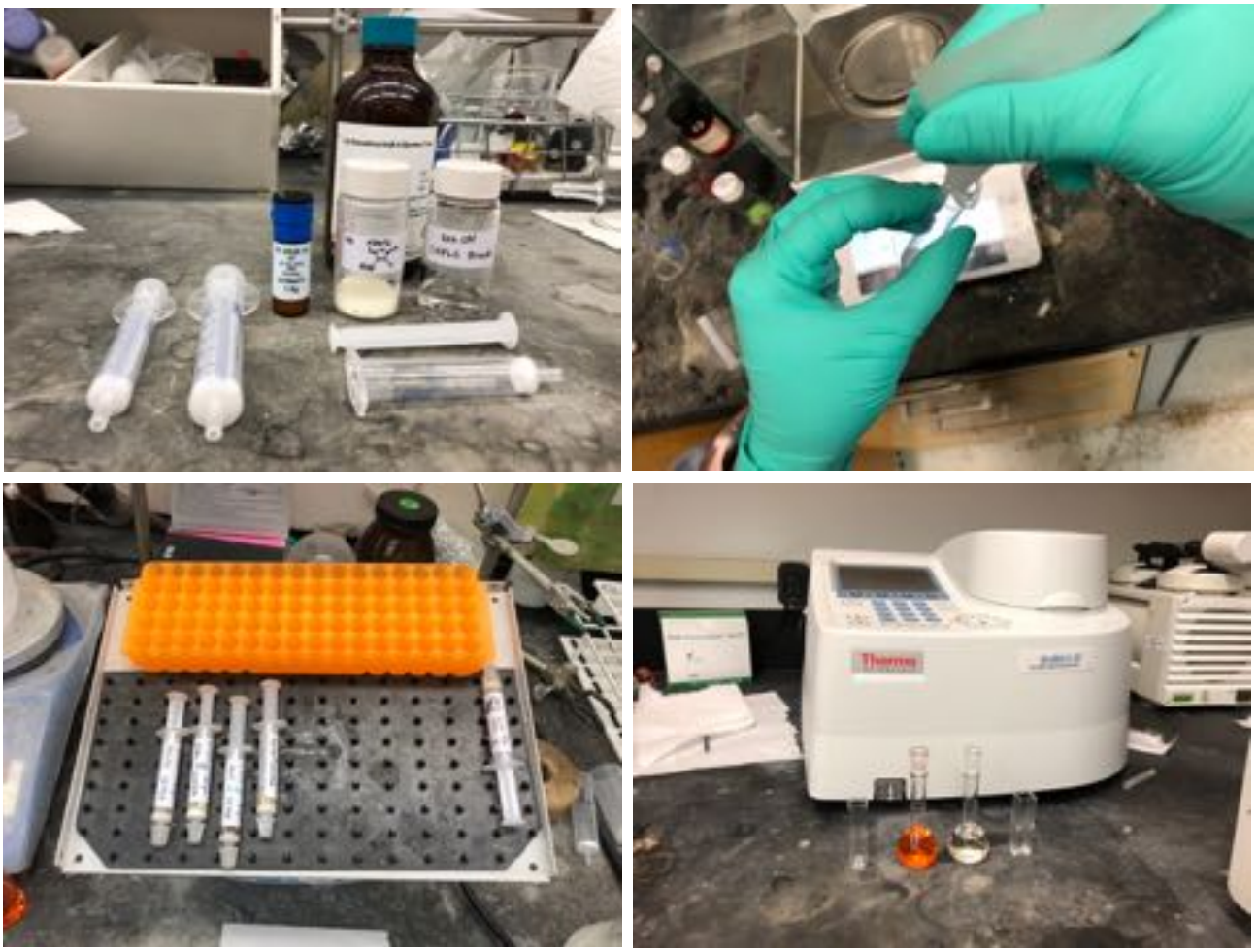
General Solid-Phase Capping

In separate vials, solutions of Ac_2O/2,6-lutidine/THF (1 mL, 10:10:80 v/v/v) and NMI/THF (1 mL, 16:84 v/v) were prepared. The resin was washed with THF (5 x 2 mL). The prepared solutions were quickly combined, added to the resin immediately (2 mL), then agitated for 1 min at room temperature. The capping solution was expelled and the resin washed with THF (5 x 2 mL), DCM (5 x 2 mL), DMF (5 x 2 mL), then DCM (5 x 2 mL). DMTr deprotection was then affected according to the *General Solid-Phase Deprotection and Efficiency Evaluation* protocol.

Cleavage

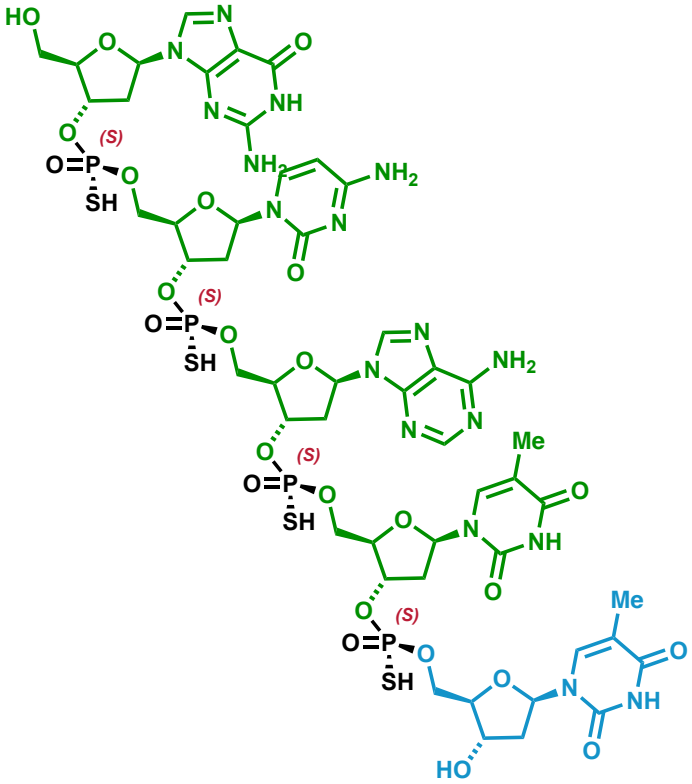
The resin was washed with H_2O (5 x 2 mL) then treated with NH_4OH (28% aq., 1 x 2 mL) and agitated for 5 min at room temperature. Following expulsion of the solution into a clean scintillation vial, the resin was wash with NH_4OH (28% aq., 3 x 2 mL) and H_2O (5 x 2 mL) to ensure complete resin cleavage. The combined cleavage solution and aqueous washes were stirred at 55 °C for 16 h to aid protecting group removal.

Pictorial Guide



(From top left, clockwise): Torviq solid-phase peptide synthesis vessel with frit, dT-Q-CPG resin, and required reagents for ψ coupling reactions; open air, dry addition of the nucleoside–P(V) coupling partner to the back of the syringe; assessment of reaction efficiency via $DMTr^+$ deprotection and UV assay; standard orbital shaker used to agitate manual SPOS reactions.

Compound SI-(S_P, S_P, S_P, S_P)-24



SI-(S_P, S_P, S_P, S_P)-24

Compound **SI-(S_P, S_P, S_P, S_P)-24** was prepared using **(R_P)-22, SI-(R_P)-14**, **SI-(R_P)-15** and **SI-(R_P)-16**.

Physical State: amorphous solid;
HRMS (ESI-TOF, m/z): Calcd for $[C_{49}H_{63}N_{17}O_{25}P_4S_4+H]^+$ 1542.2093; Found 15

Troubleshooting and Frequently Asked Questions

Reagent Synthesis.
Question 1: How do you monitor the reactions?
Answer: Reaction progress is monitored by taking a small aliquot and analyzing by LCMS and ^{31}P NMR.

Question 2: Do I need to use isomerically pure *cis*-limonene oxide?
Answer: Use of the commercial (~1:1, cis/trans) mixture of limonene oxide results in a mixture of ψ reagents that are difficult to isolate in pure form. However, we have used ~9:1, cis/trans mixtures from the resolution step with no difficulty. The reagents derived from *trans*-limonene oxide were found to exhibit drastically different reactivity.

Question 3: My synthesis of ψ didn't give me a single diastereomers, can I use this material anyways for loading and coupling?
Answer: The *d.r* at phosphorus translates from ψ to the loaded intermediates and finally to the coupled compounds, this means that a 1:1 mixture of ψ will give you a 1:1 mixture of coupled phosphorothioate dinucleotides. If high *d.r* is desired, we recommend a recrystallization of the reagent (see **Synthesis of ψ Reagents** for more details).

Question 4: Do any special precautions need to be taken when setting up the reactions?
Answer: Standard reaction set up includes the use of inert atmosphere and flame dried glassware. However, there is no effect seen if these precautions are not followed.

Note: During the synthesis of Compound 3, P_2S_5 releases H_2S upon contact with moisture, although minimal, care should be taken on large scale to avoid contact with reaction mixtures/waste outside of a fume hood.

Question 5: How do you store ψ reagents? Do any special precautions need to be taken in the storage of this reagent?
Answer: The ψ reagents are bench-stable, and no special precautions need be taken when dry. Although the reagents are stable to aqueous work up, they are susceptible to base catalyzed (pH >7) hydrolysis in a water miscible solvent (THF, MeCN, MeOH, etc.). Anhydrous solutions of the reagents in MeCN are indefinitely stable at ambient temperature.

Loading/Coupling Reactions.
Question 1: How do you monitor the reactions?
Answer: The crude reaction is either monitored by thin layer chromatography or by RP-HPLC.

Question 2: How do you purify the crude reaction mixture?
Answer: The crude reaction mixture is either purified by flash column chromatography or by RP-HPLC.

Question 3: What are the major side products observed?

Answer: Reaction side products include hydrolysis of the ψ reagent, hydrolysis of ψ-loaded compounds, and pentafluorothiophenol byproducts (S_NAr, dimerization, polymerization). If problematic, these byproducts can be minimized by using anhydrous solvent/DBU and by running the reaction at 0 °C.

Question 4: Do any special precautions need to be taken when setting up the reactions?
Answer: Standard reaction set up includes the use of inert atmosphere and flame dried glassware. There is no effect seen if these precautions are not followed when using excess (~1.3 equiv.) of ψ reagent.

Solid-Phase Synthesis.
Question 1: How do you assess reaction cycle efficiency?
Answer: Efficiency of a reaction cycle (i.e., couple-cap-deprotect) can be assessed by a standard $DMTr^+$ UV assay, as described in the *General Solid-Phase Deprotection and Efficiency Evaluation* protocol. The theoretical maximum for the reported UV yields are based on the numerical value obtained in each preceding coupling reaction.

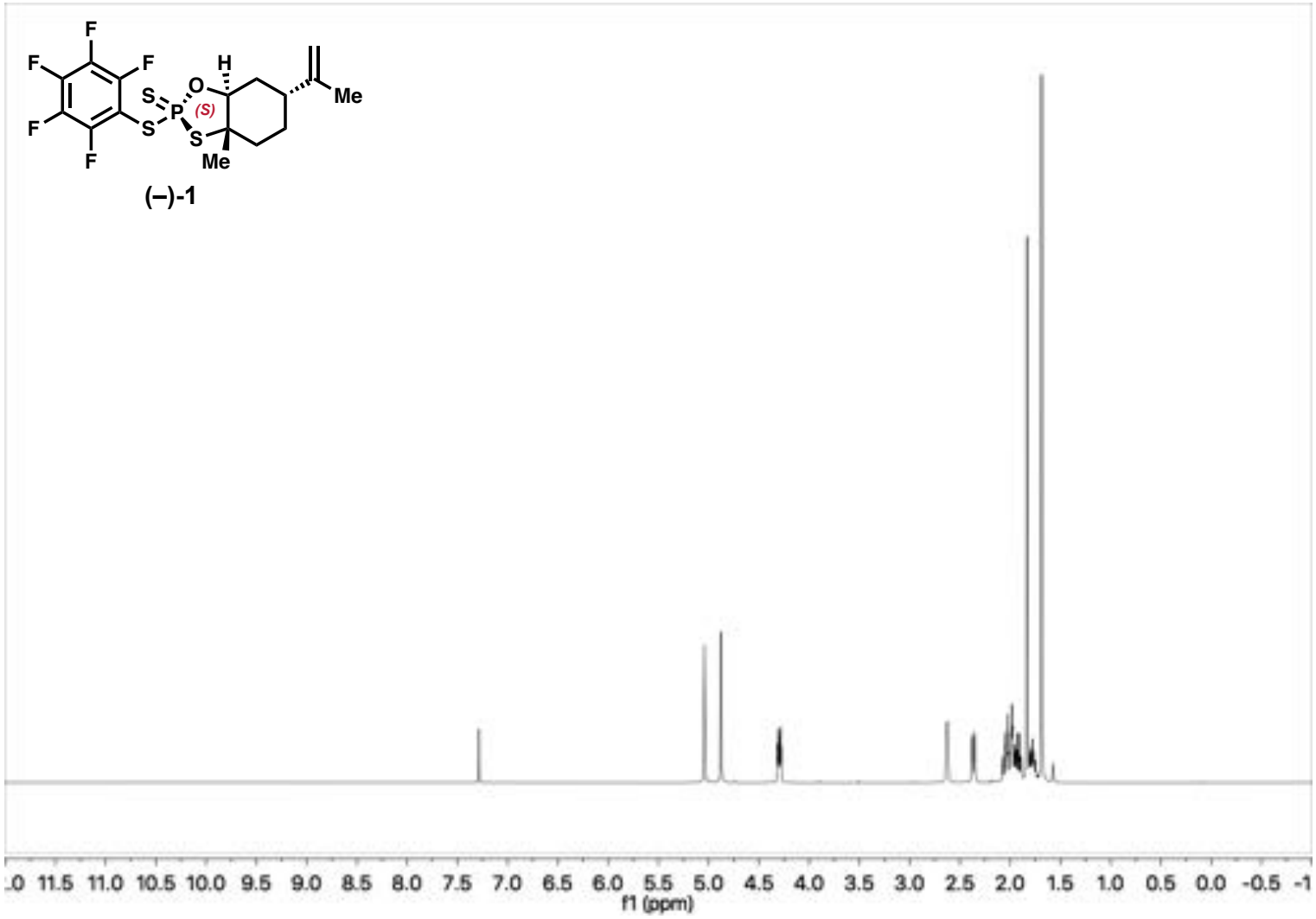
Question 2: Can I prepare a single stock solution of the nucleoside–P(V) and DBU for use in SPOS?
Answer: We recommend against the preparation of a single stock solution of nucleoside–P(V) and DBU—if stored together, rapid hydrolysis of the coupling partner is observed. When kept separate, however, stock solutions are an acceptable option. Note that the DBU solution should be prepared fresh every 24 h. While solutions of nucleoside–P(V) can be prepared for automated SPOS, hydrolysis may be observed if the solution is left open to air; it is therefore recommended that the solution be kept under inert atmosphere and made fresh every 12 h. If performing manual SPOS, we recommend adding the coupling partner dry to the back of the syringe, as described in the *General Solid-Phase Loading of ψ-Compounds* protocol.

Question 3: Must I purchase an automated oligonucleotide synthesizer to perform ψ-SPOS?
Answer: No! The protocols are readily translated to manual synthesis. See **Manual Solid-Phase Oligonucleotide Synthesis** and the accompanying pictorial guide for full details.

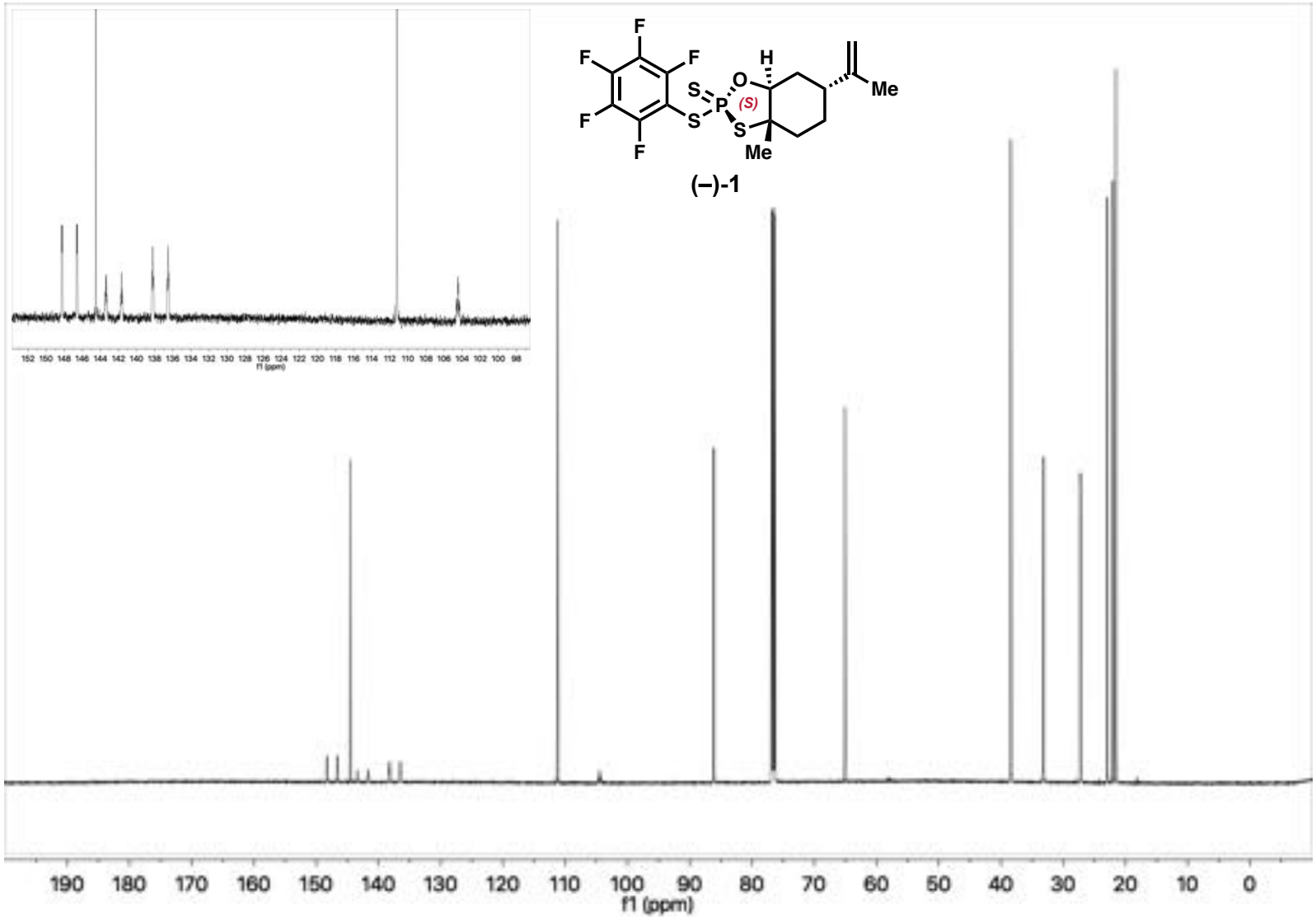
Question 4: Can I use the automated oligonucleotide synthesizer we already have?
Answer: Though designed for P(III) chemistry, all automated chemistry described was performed used the BioAutomation MerMade MM12. A bit of creativity when programming the methods should allow one to repurpose any standard instrument (provided the instrument allows for program modifications).

NMR Spectra

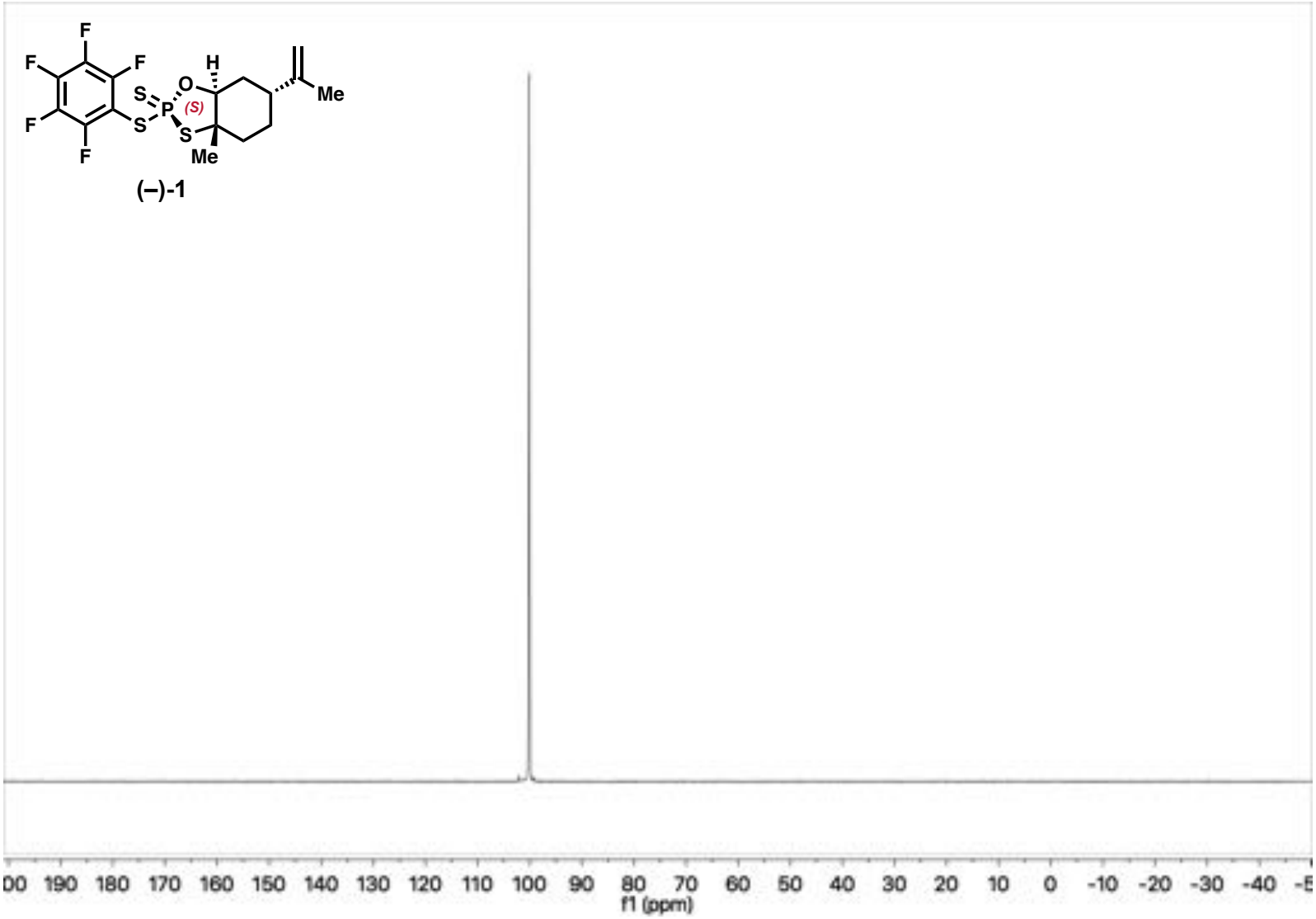
Compound (–)-1 ^{1}H NMR



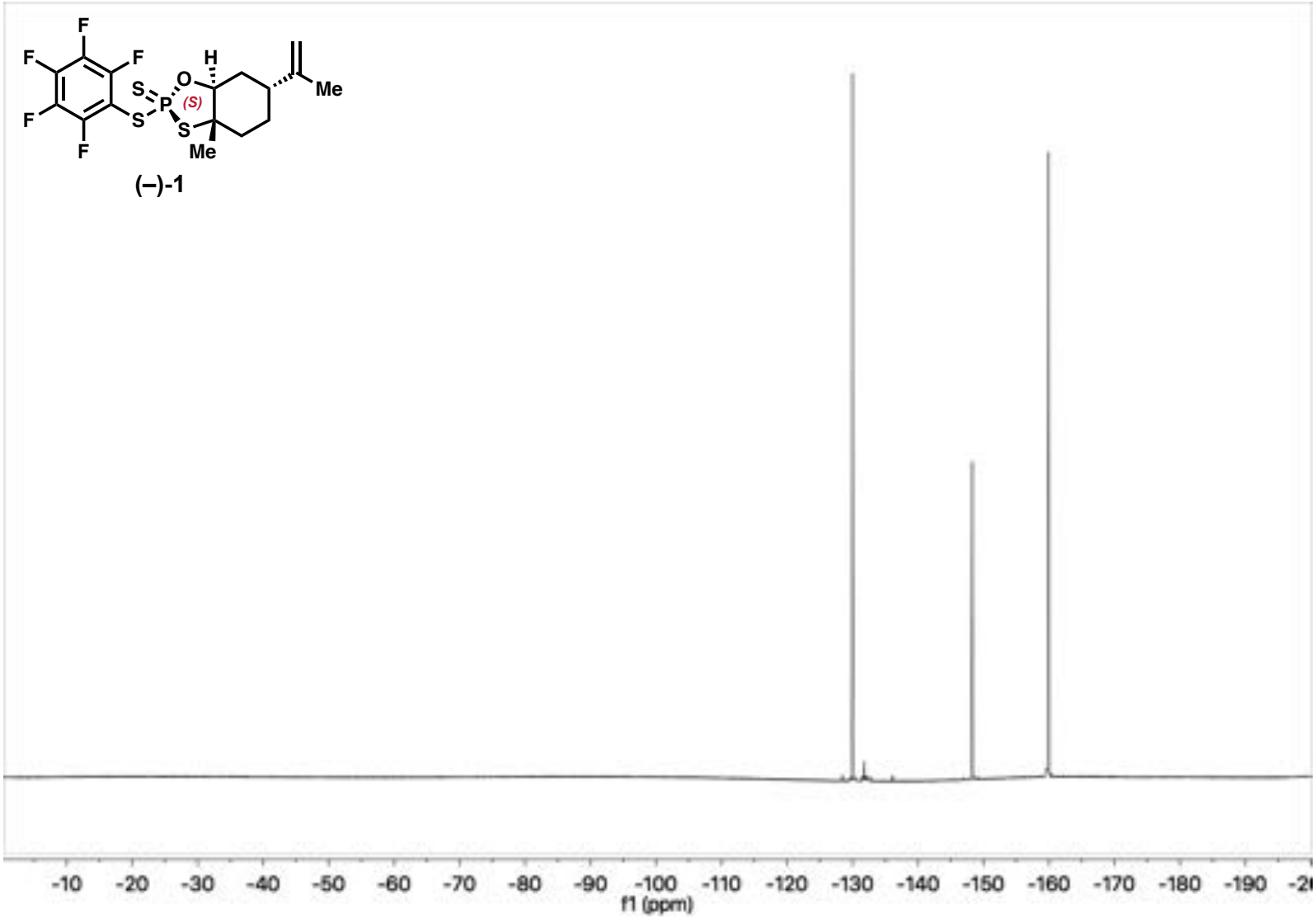
Compound (–)-1 ^{13}C NMR



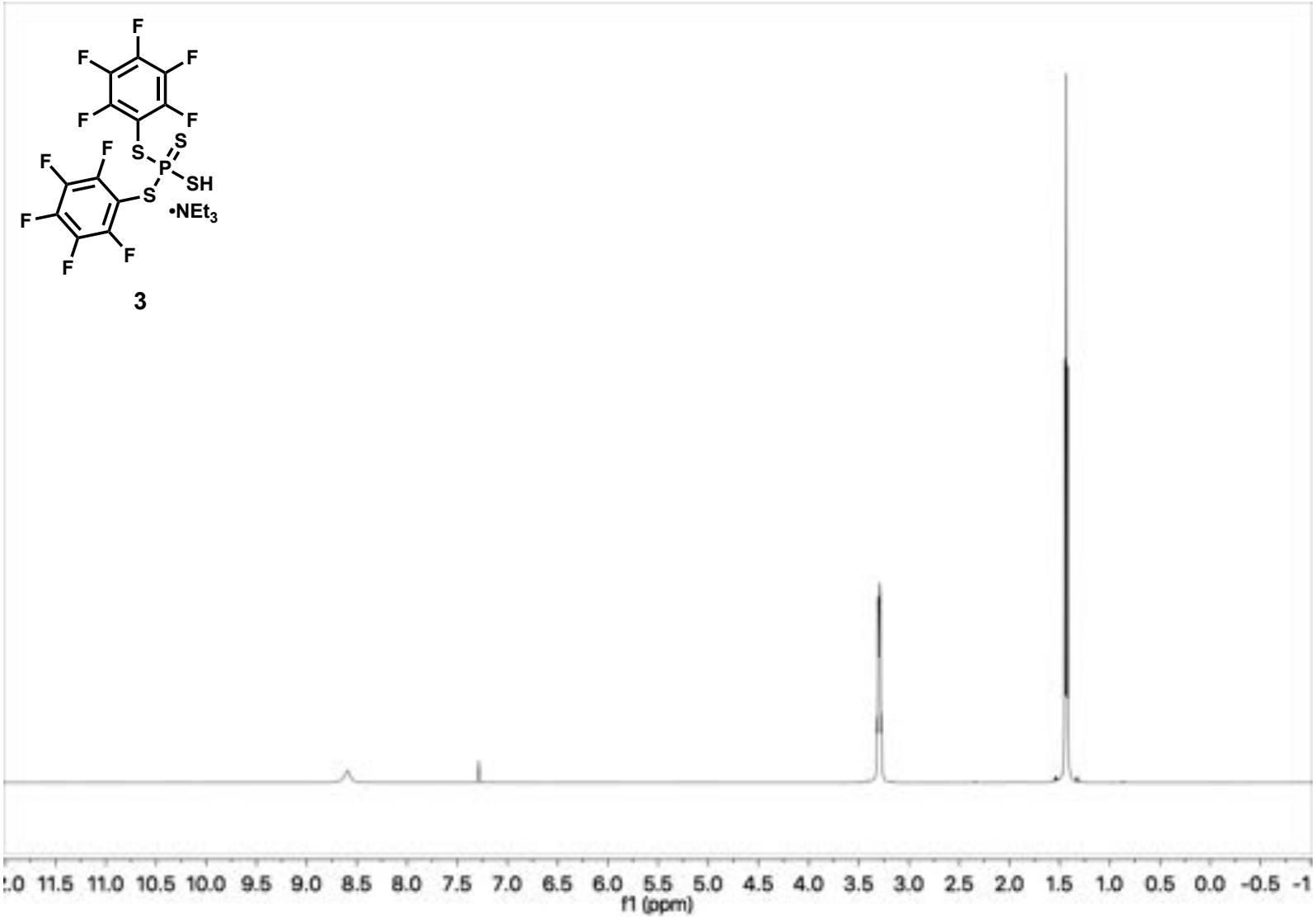
Compound (–)-1 ^{31}P NMR



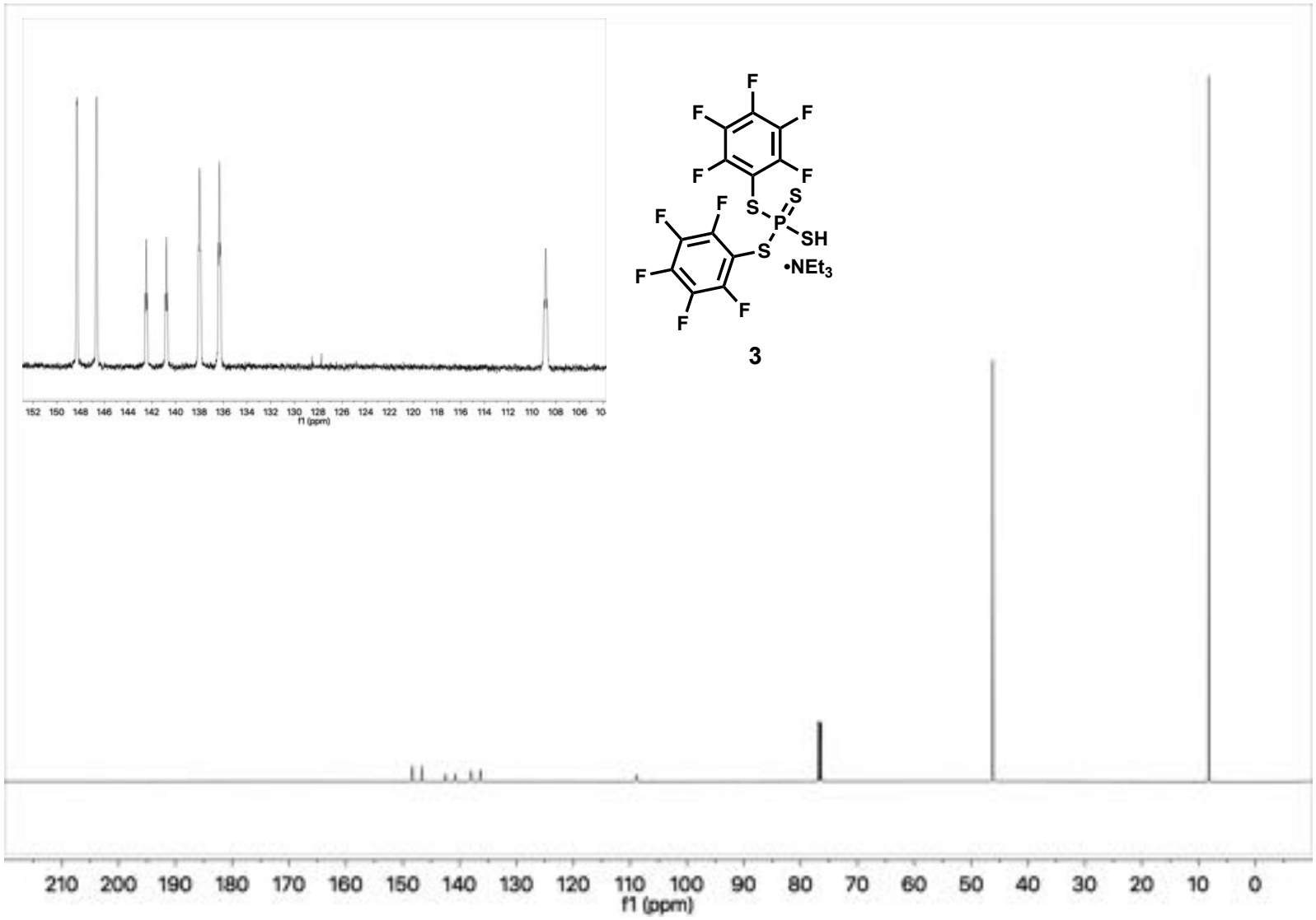
Compound (–)-1 ^{19}F NMR



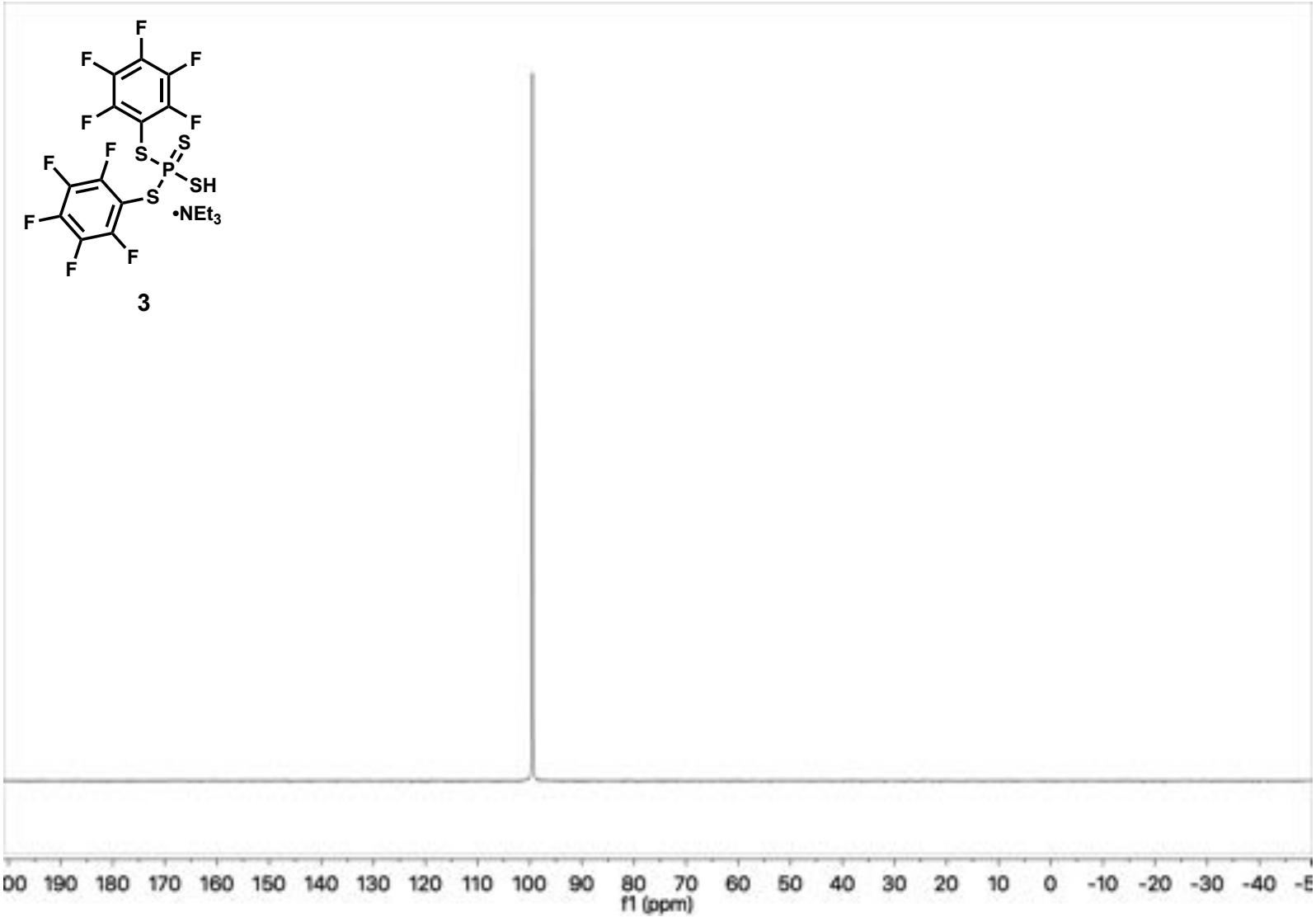
Compound 3 ^{1}H NMR



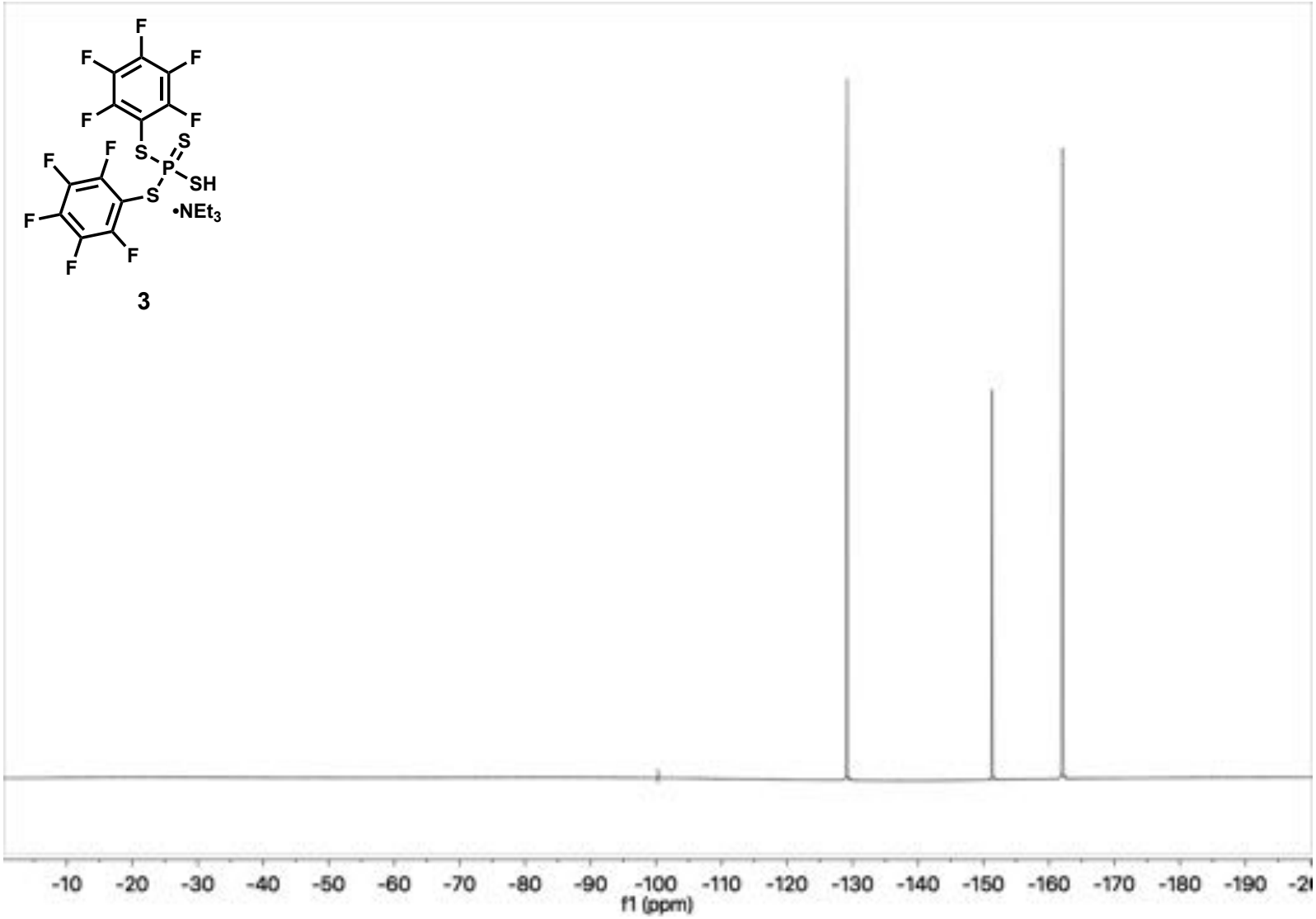
Compound 3 ^{13}C NMR



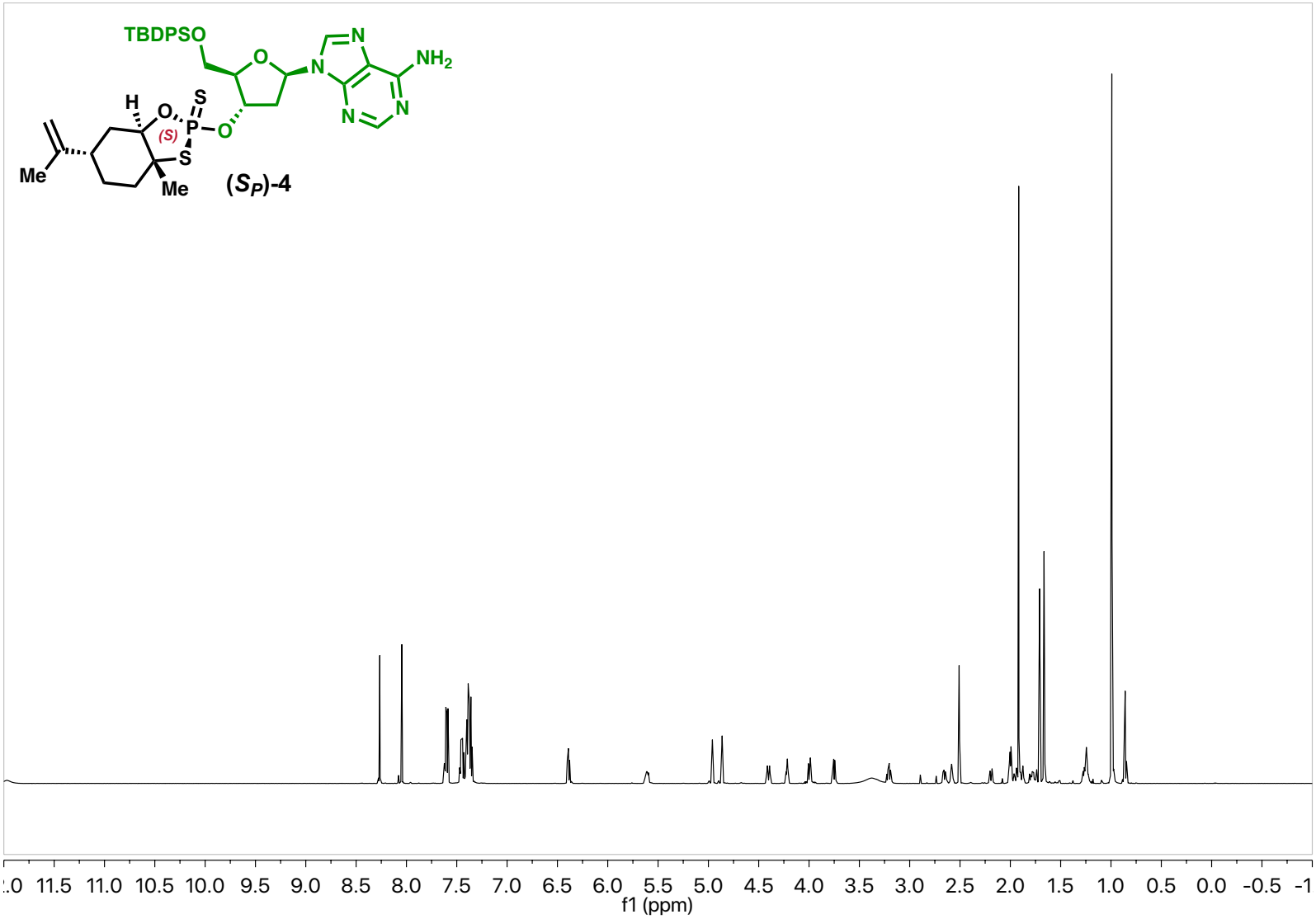
Compound 3 ^{31}P NMR



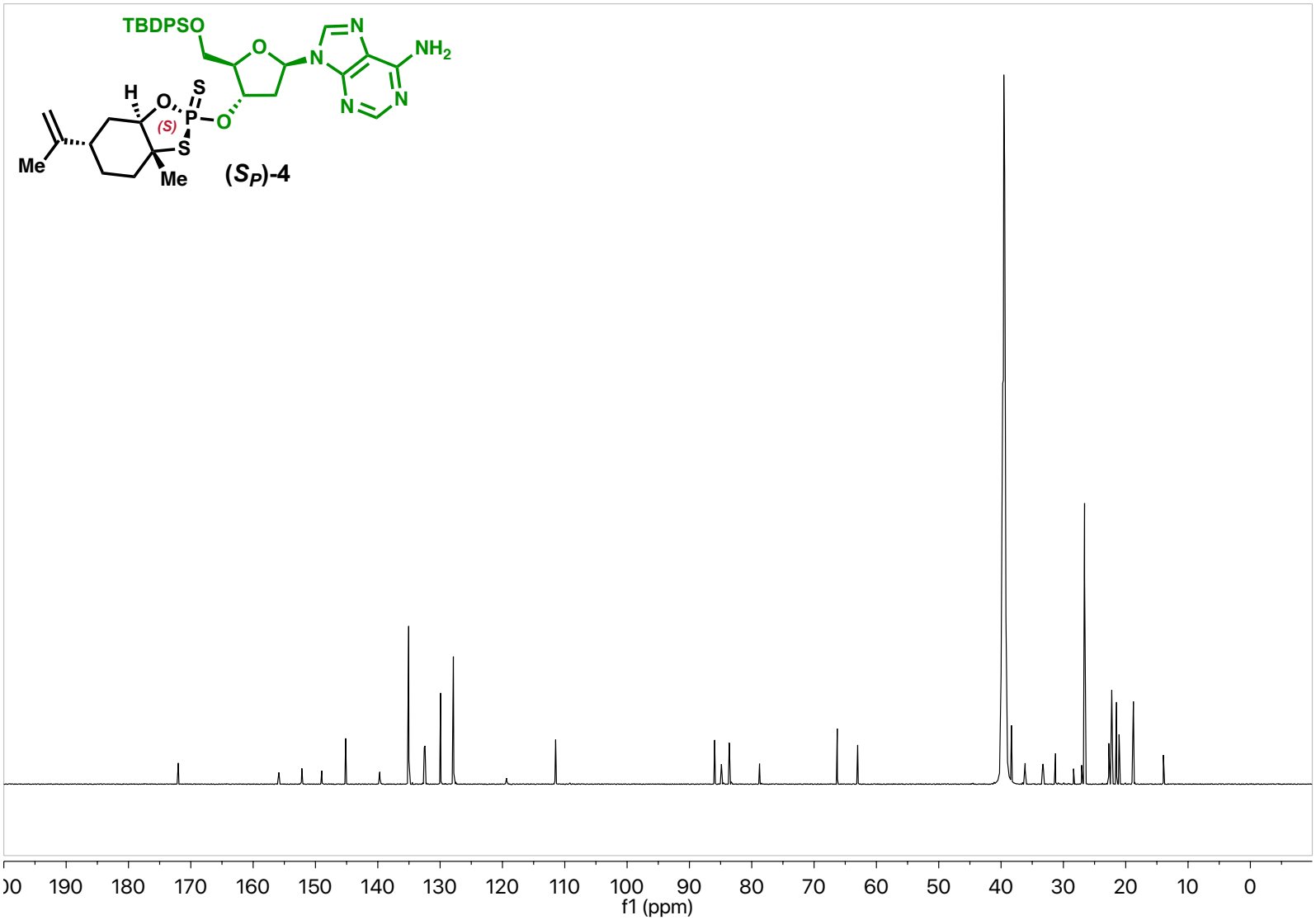
Compound 3 ^{19}F NMR



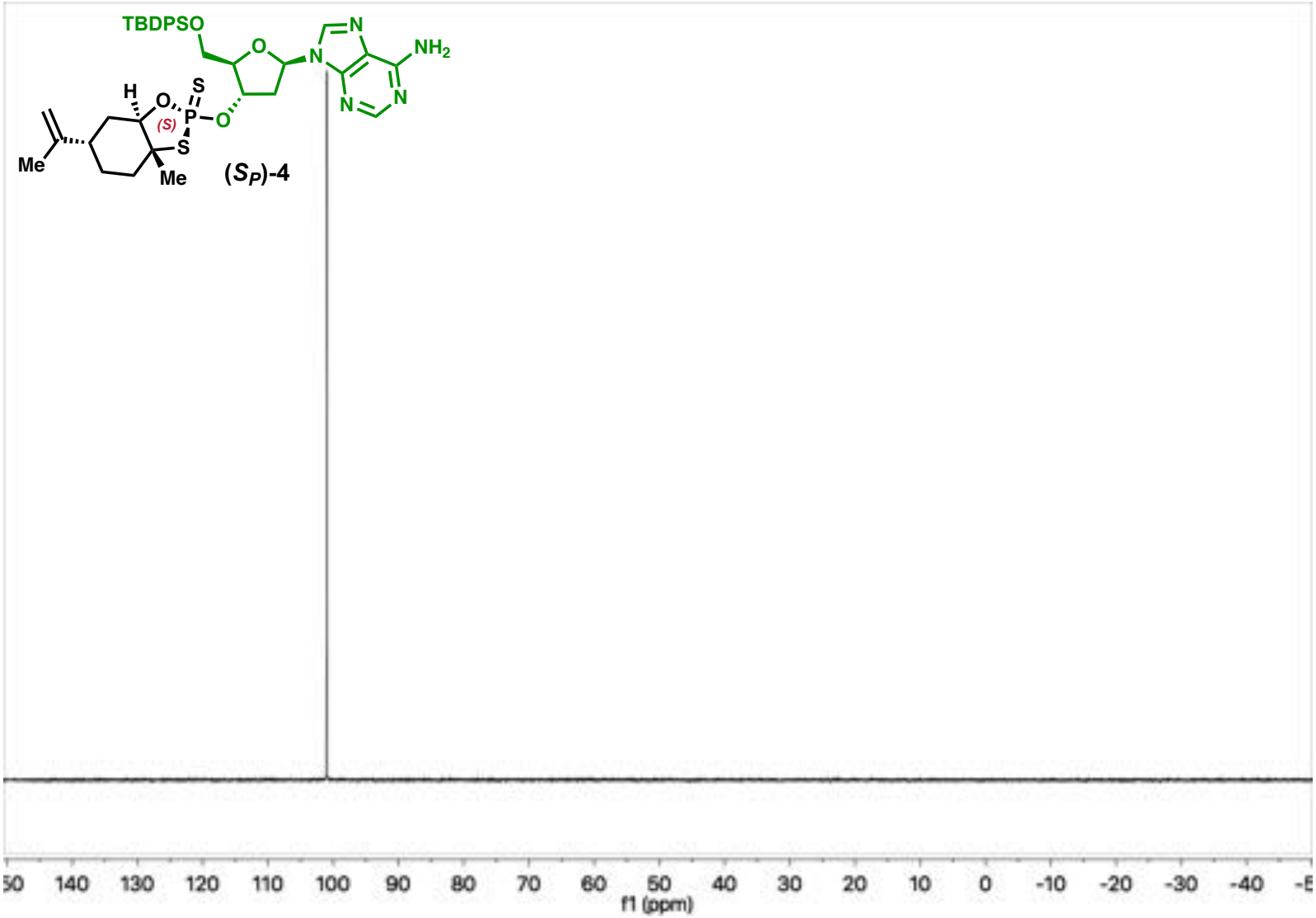
Compound (S_P)-4 ^{1}H NMR



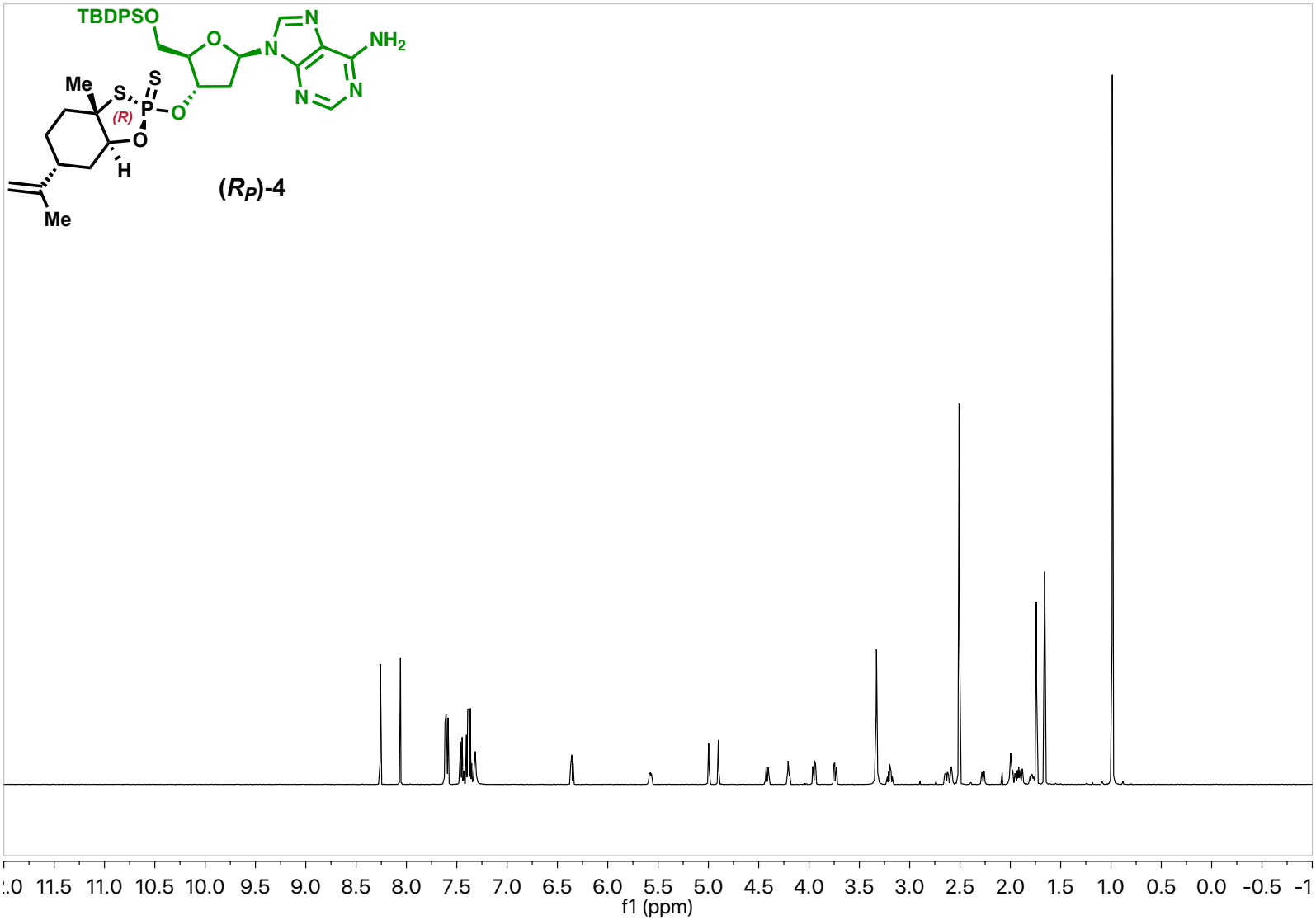
Compound (S_P)-4 ^{13}C NMR



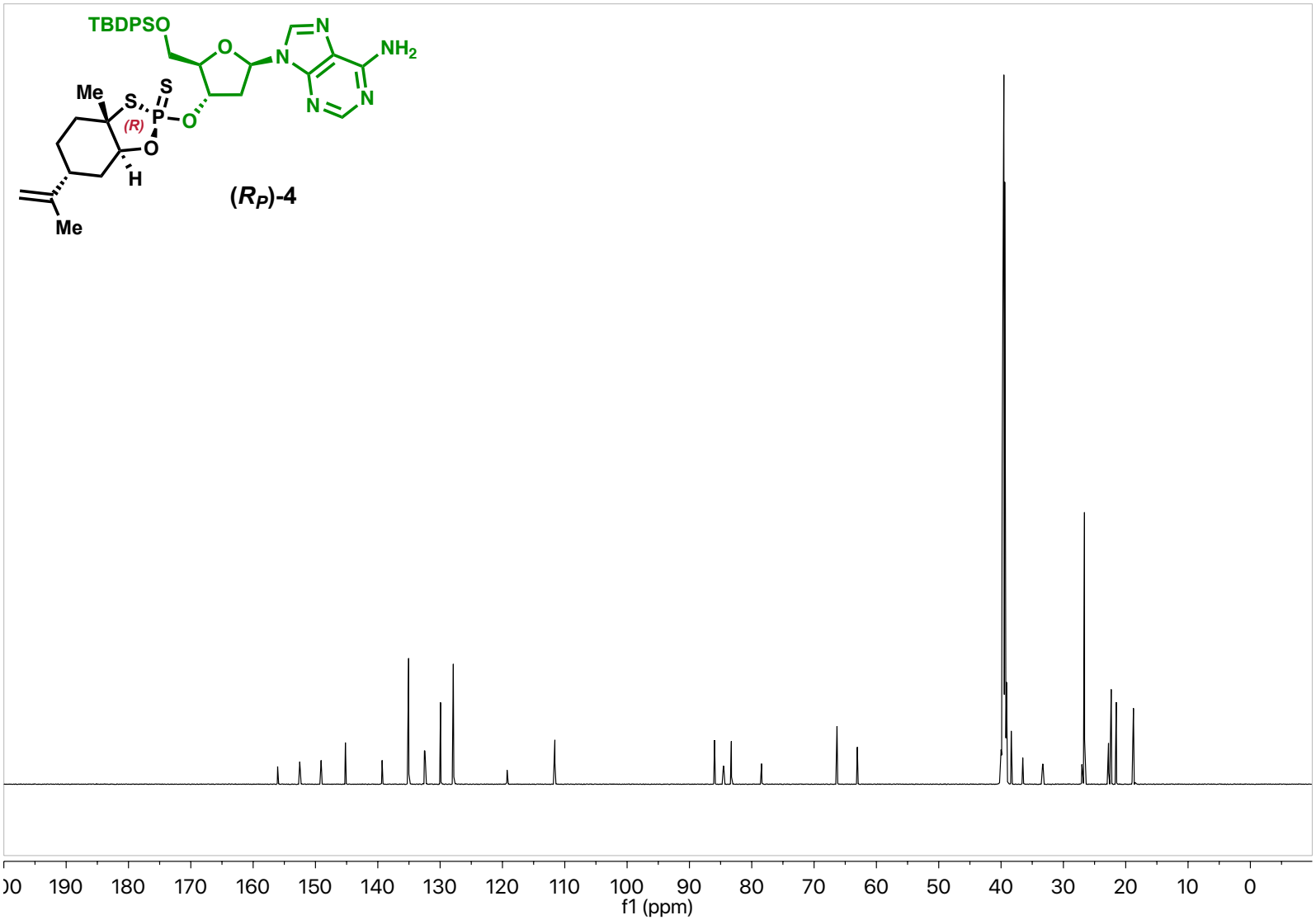
Compound (S_P)-4 ^{31}P NMR



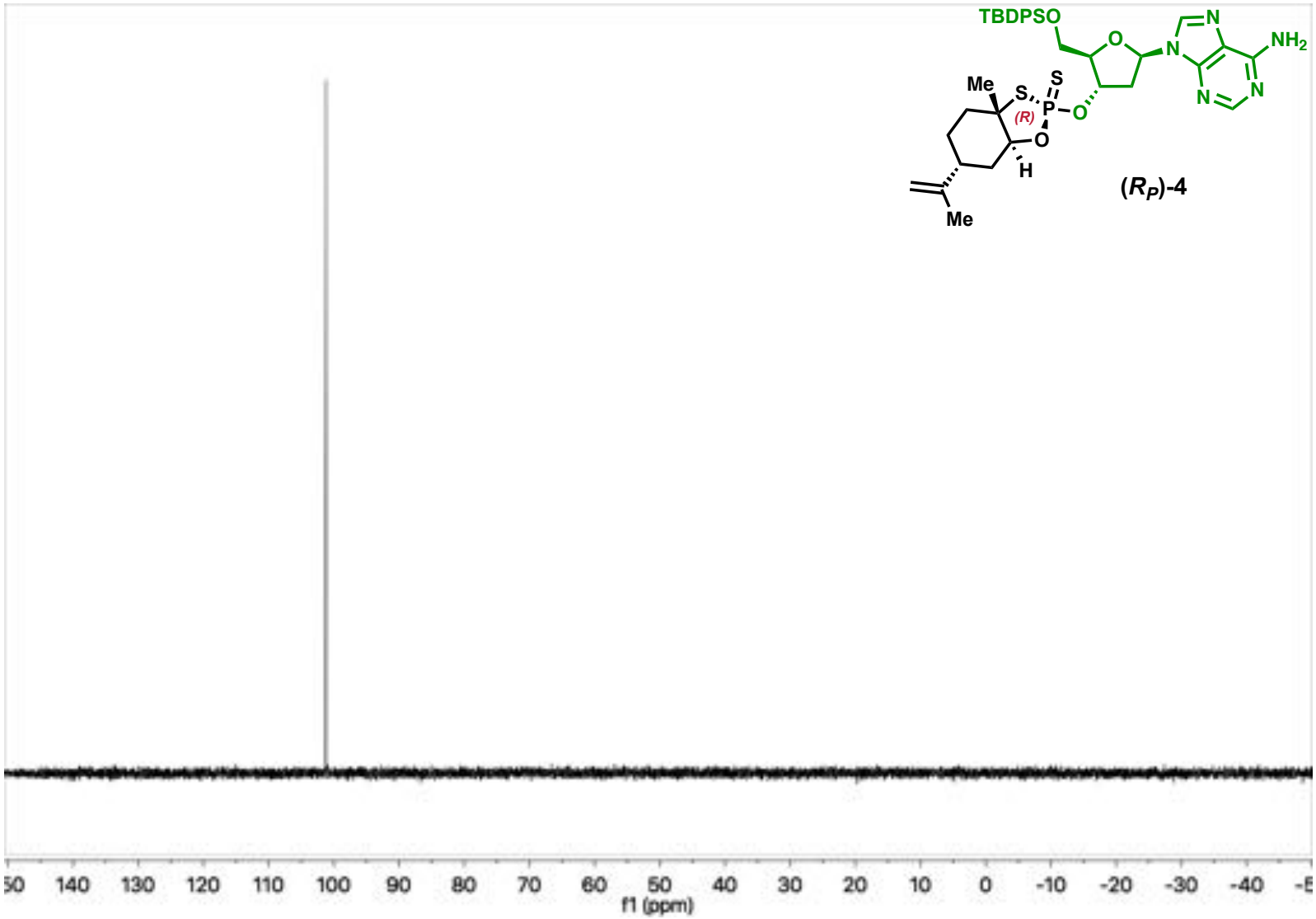
Compound (R_P)-4 ^{1}H NMR



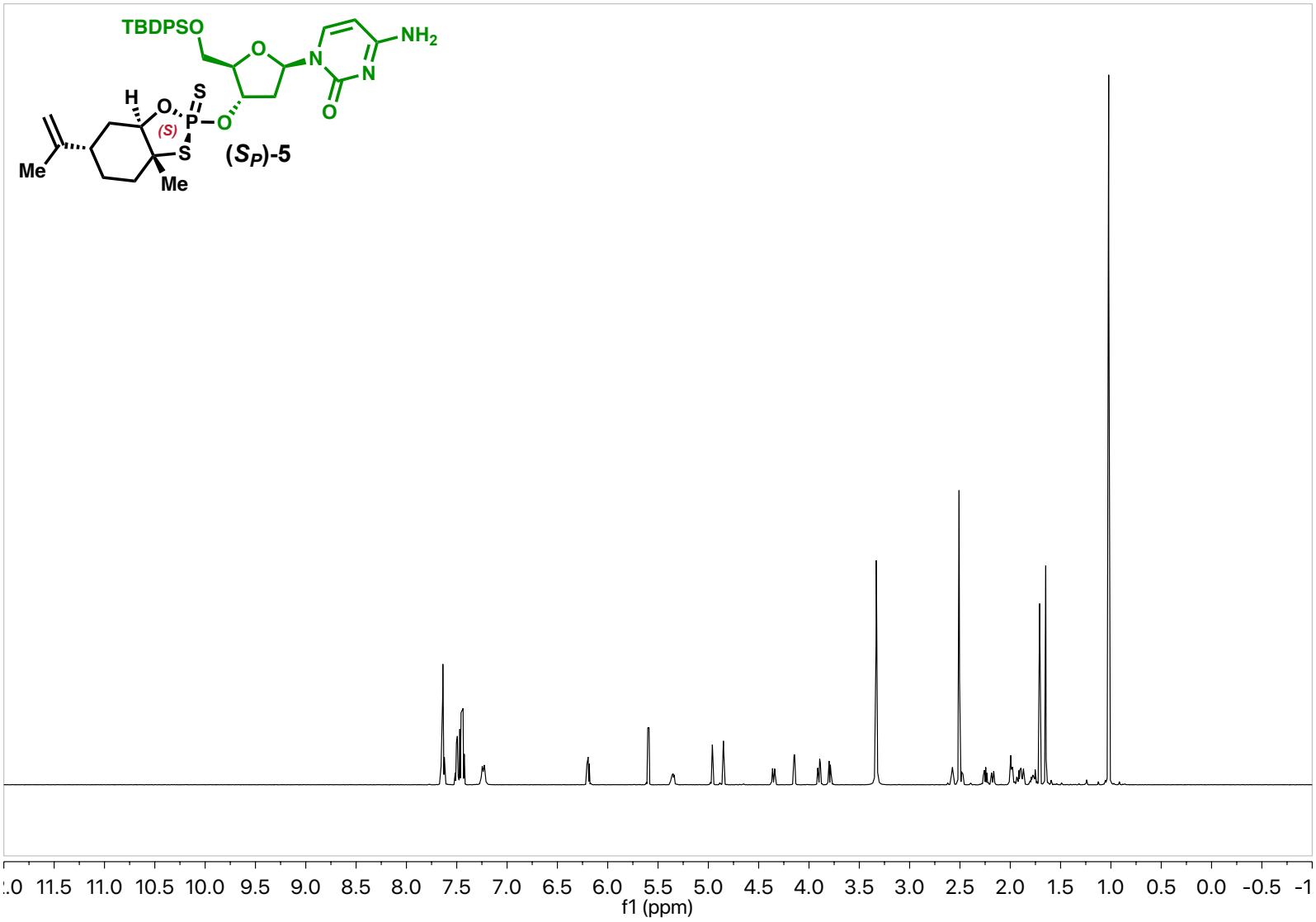
Compound (R_P)-4 ^{13}C NMR



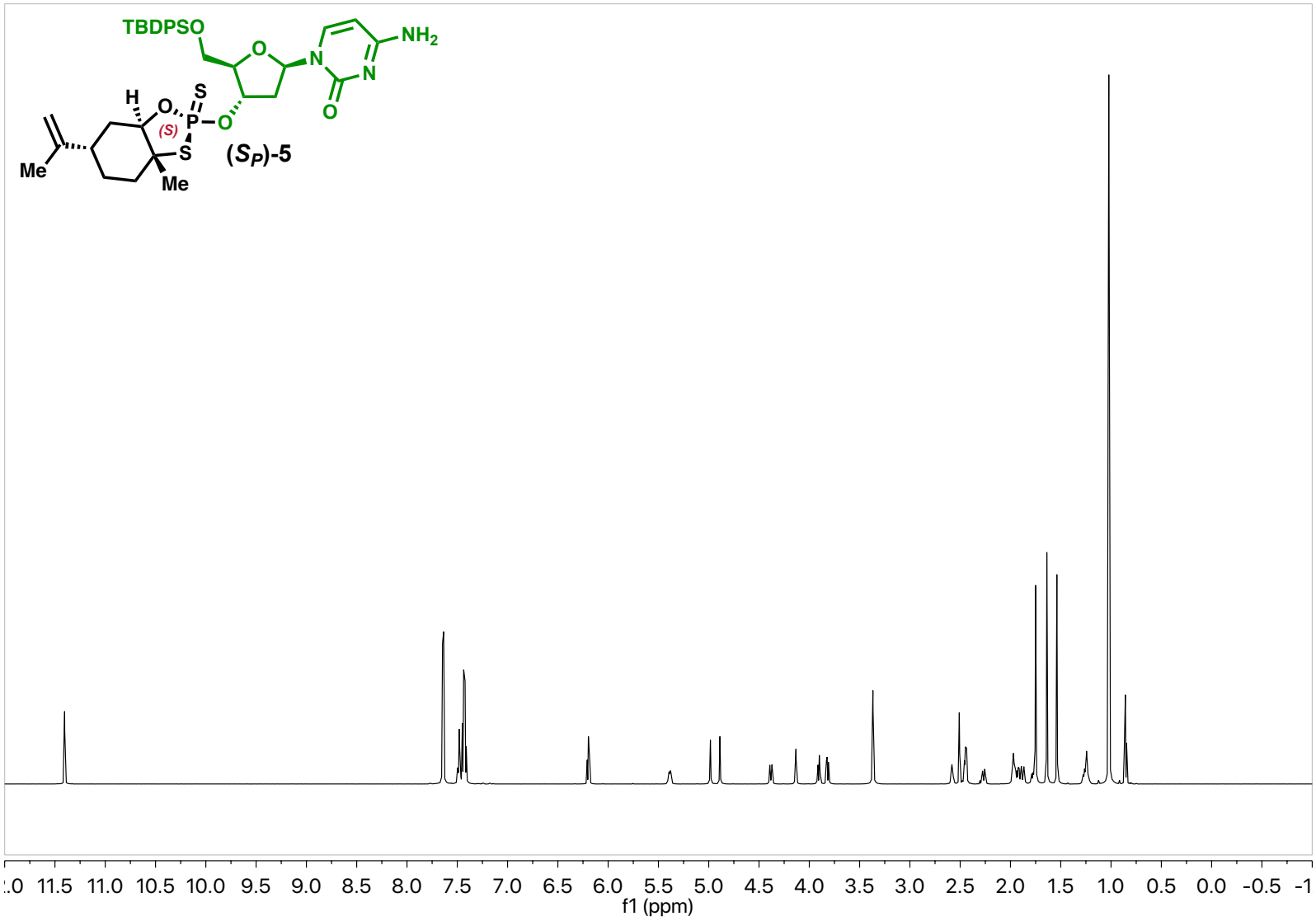
Compound (R_P)-4 ^{31}P NMR



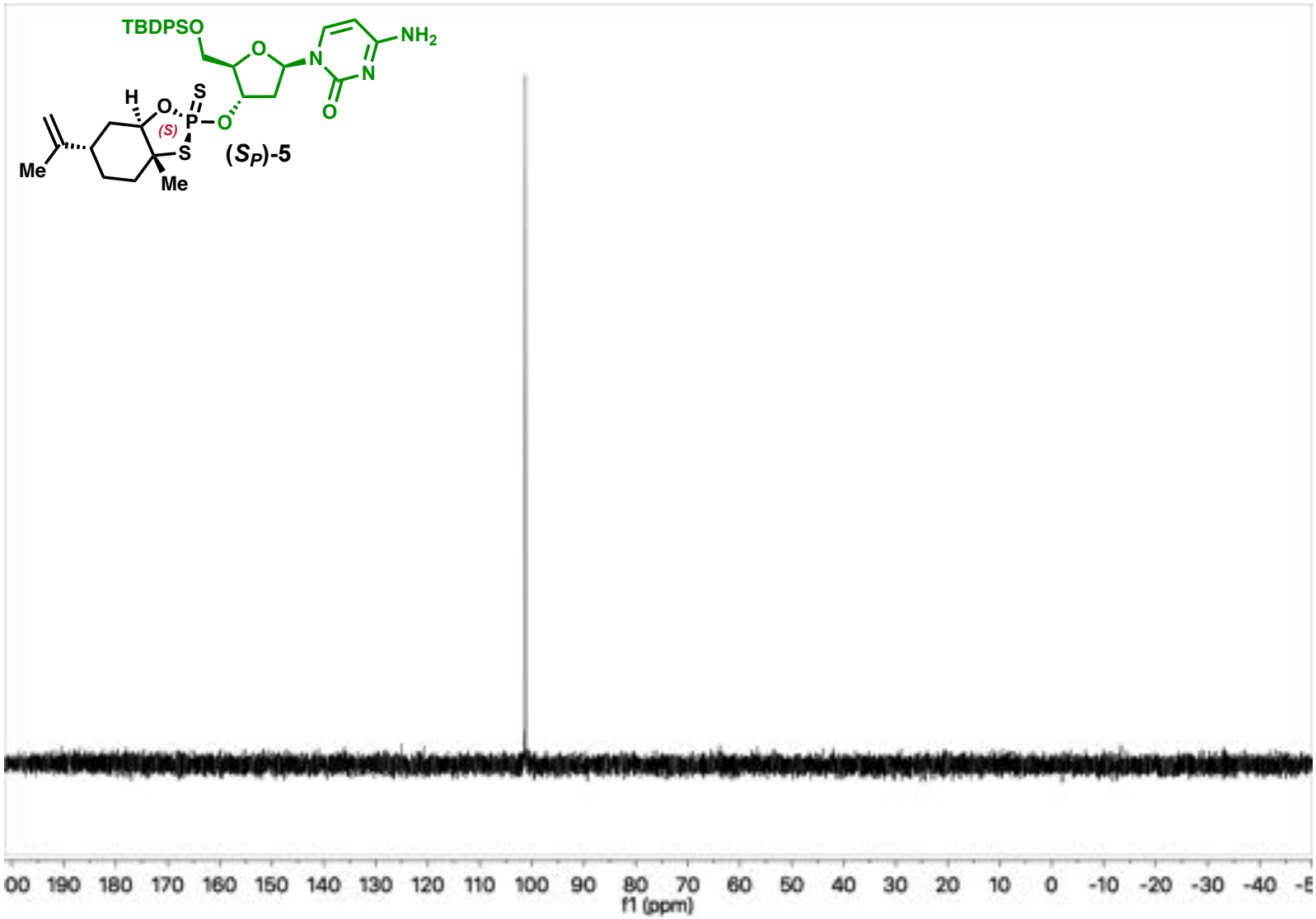
Compound (S_P)-5 ^{1}H NMR



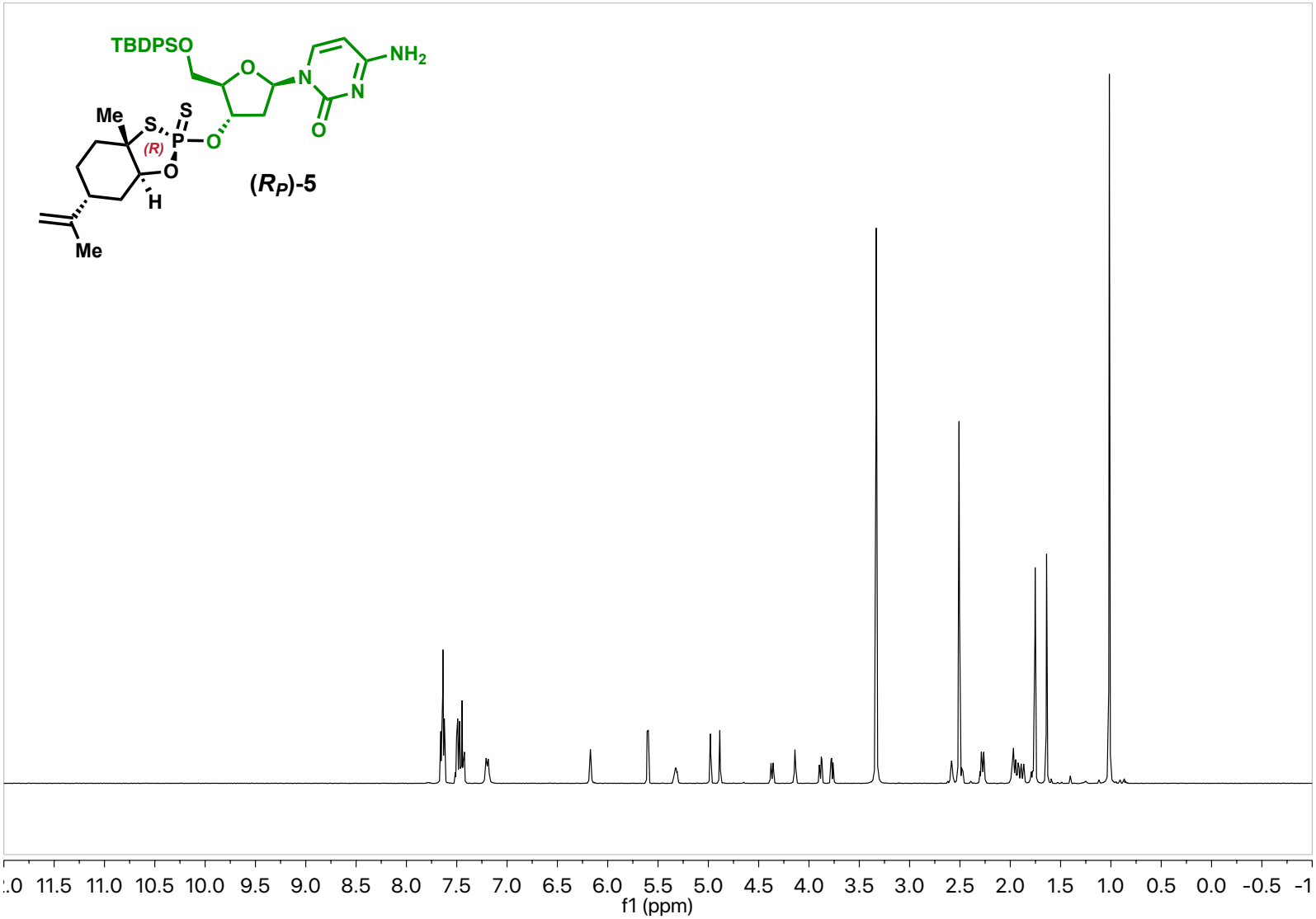
Compound (S_P)-5 ^{13}C NMR



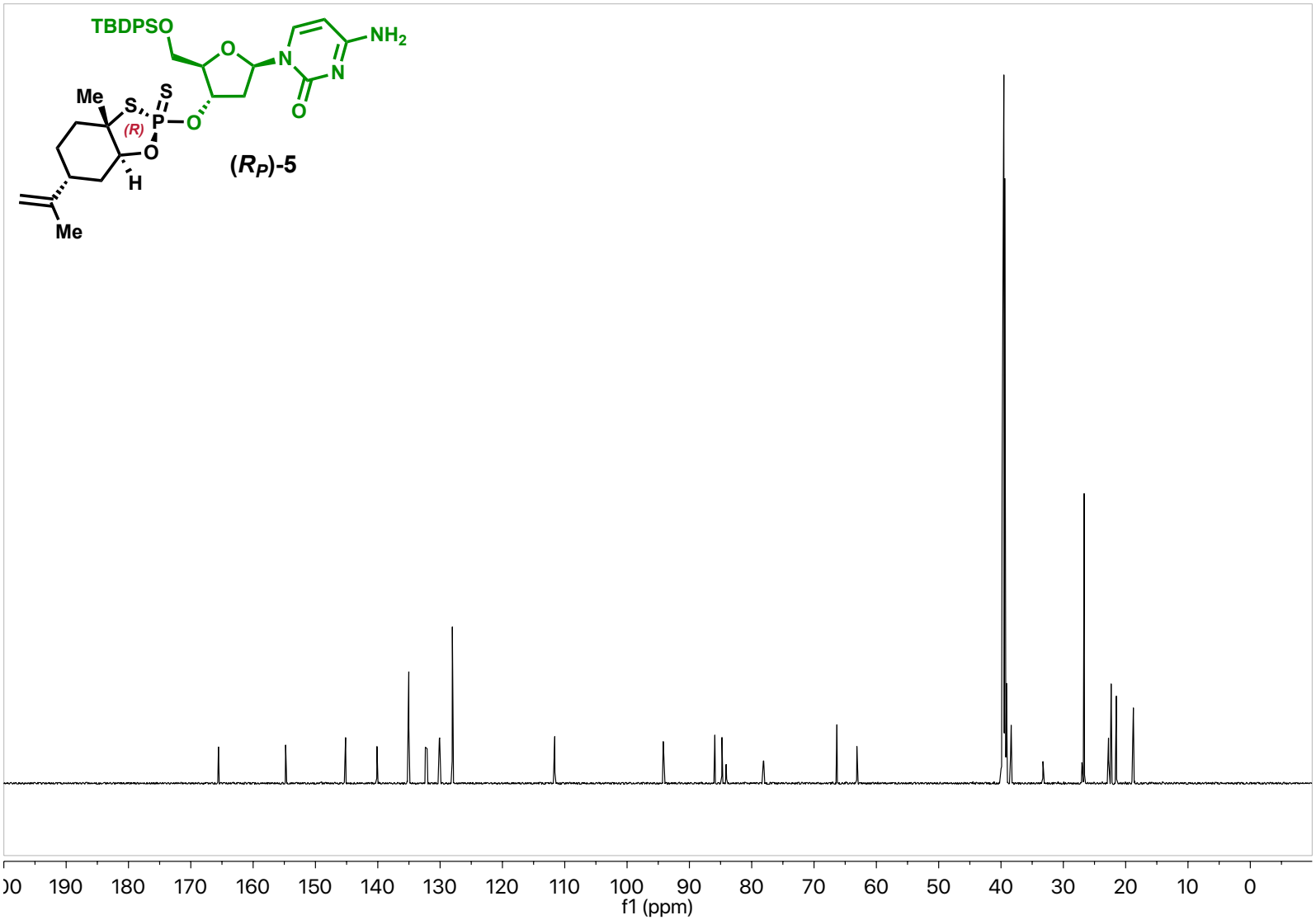
Compound (S_P)-5 ^{31}P NMR



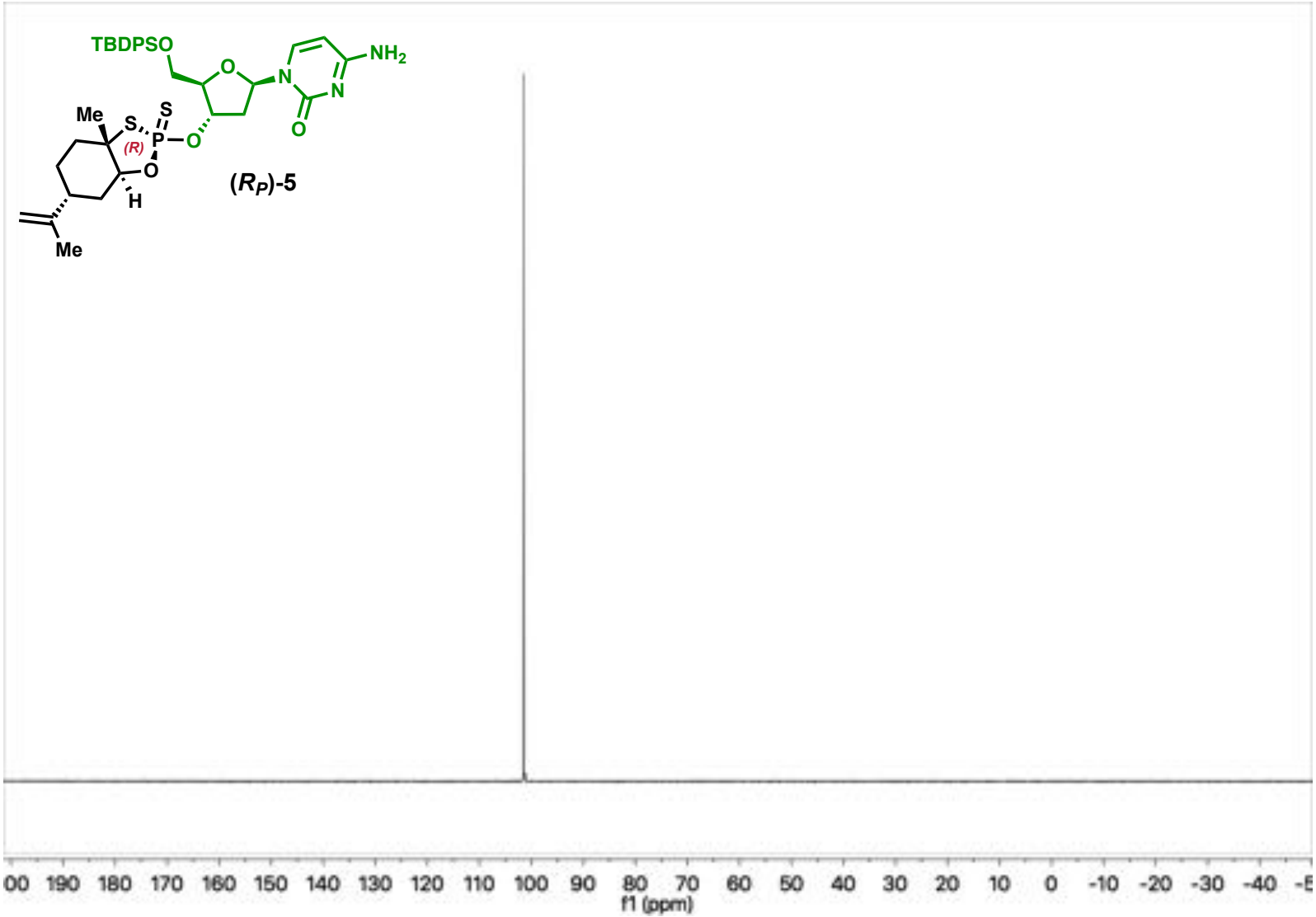
Compound (R_P)-5 ^{1}H NMR



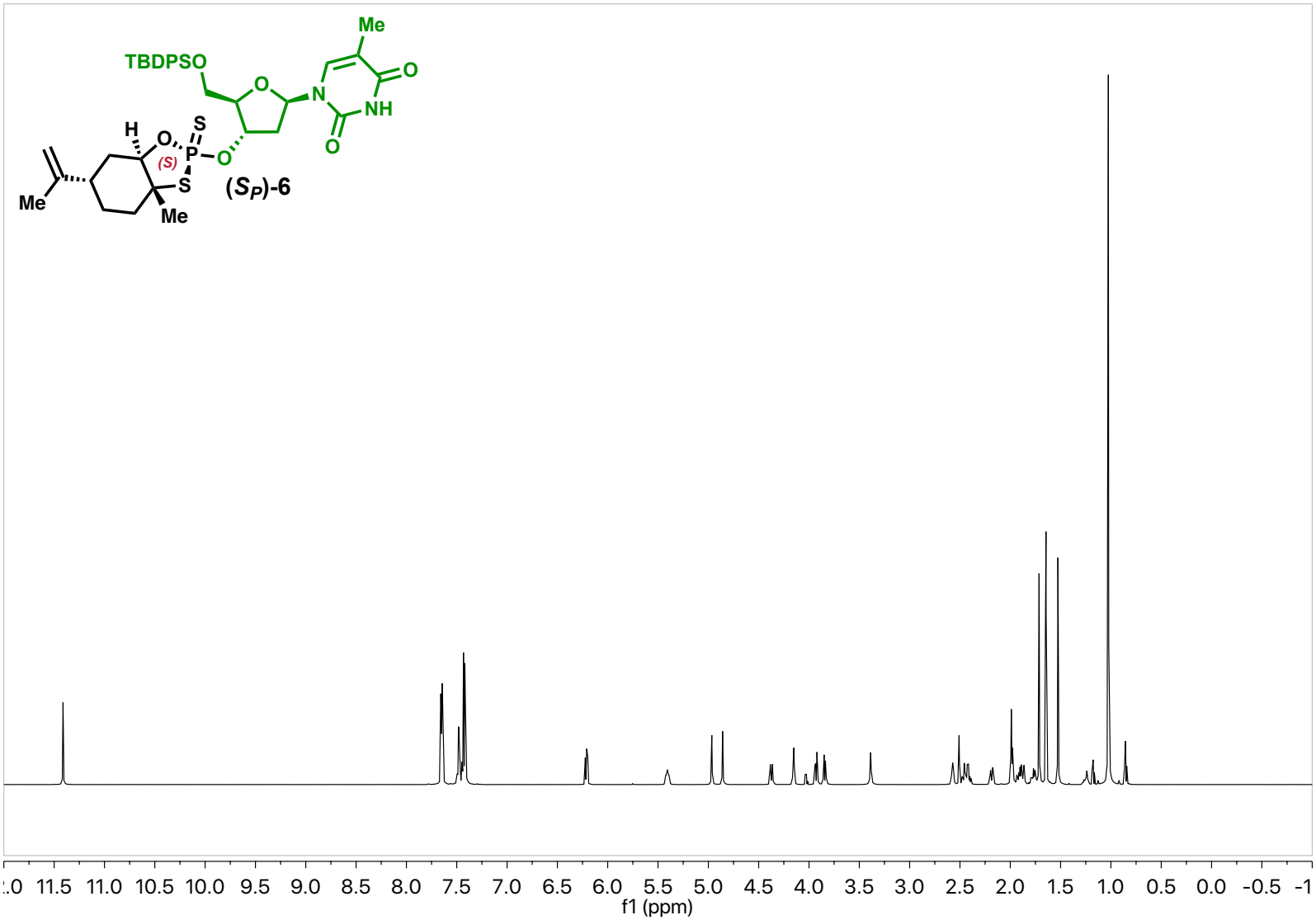
Compound (R_P)-5 ^{13}C NMR



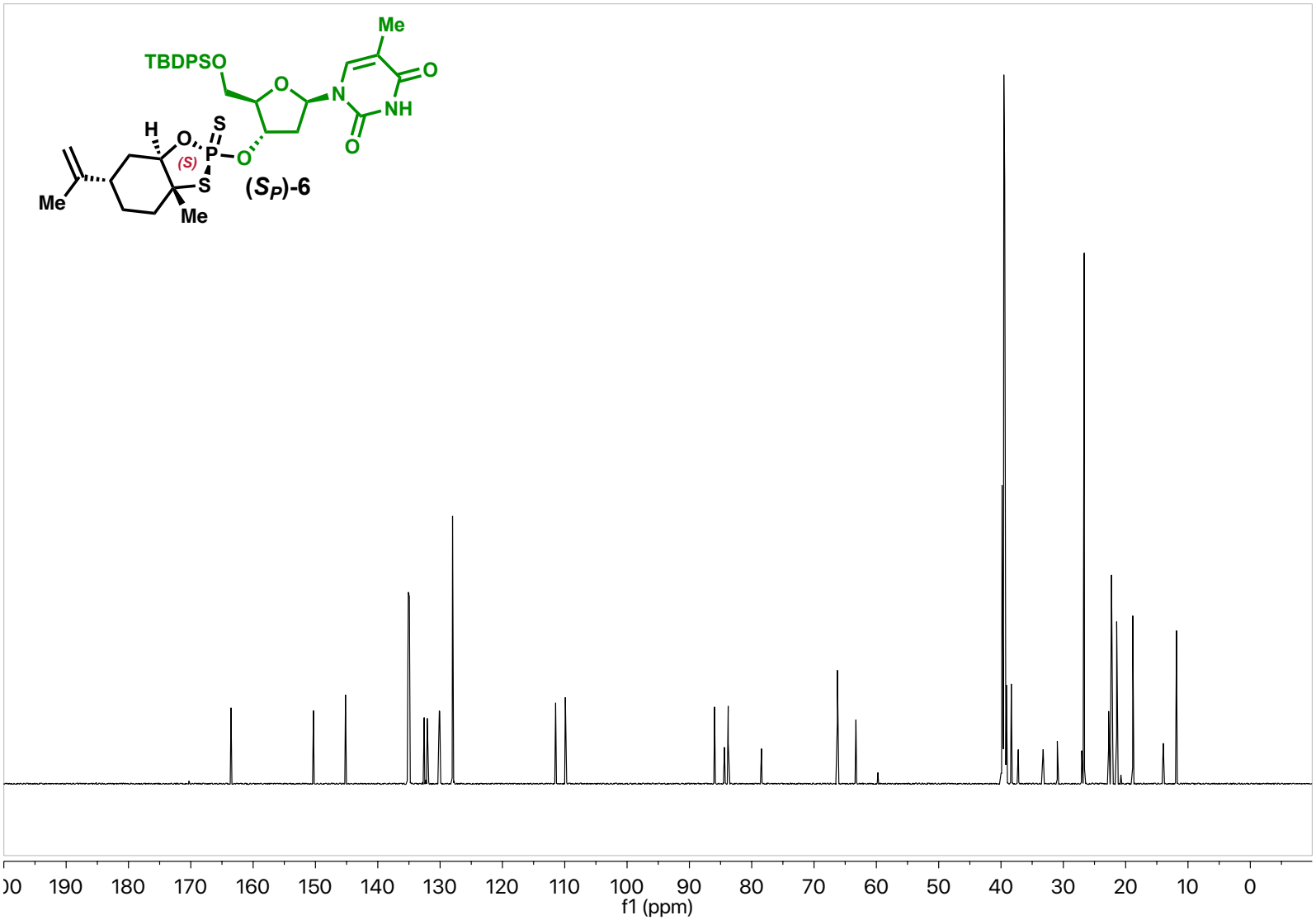
Compound (R_P)-5 ^{31}P NMR



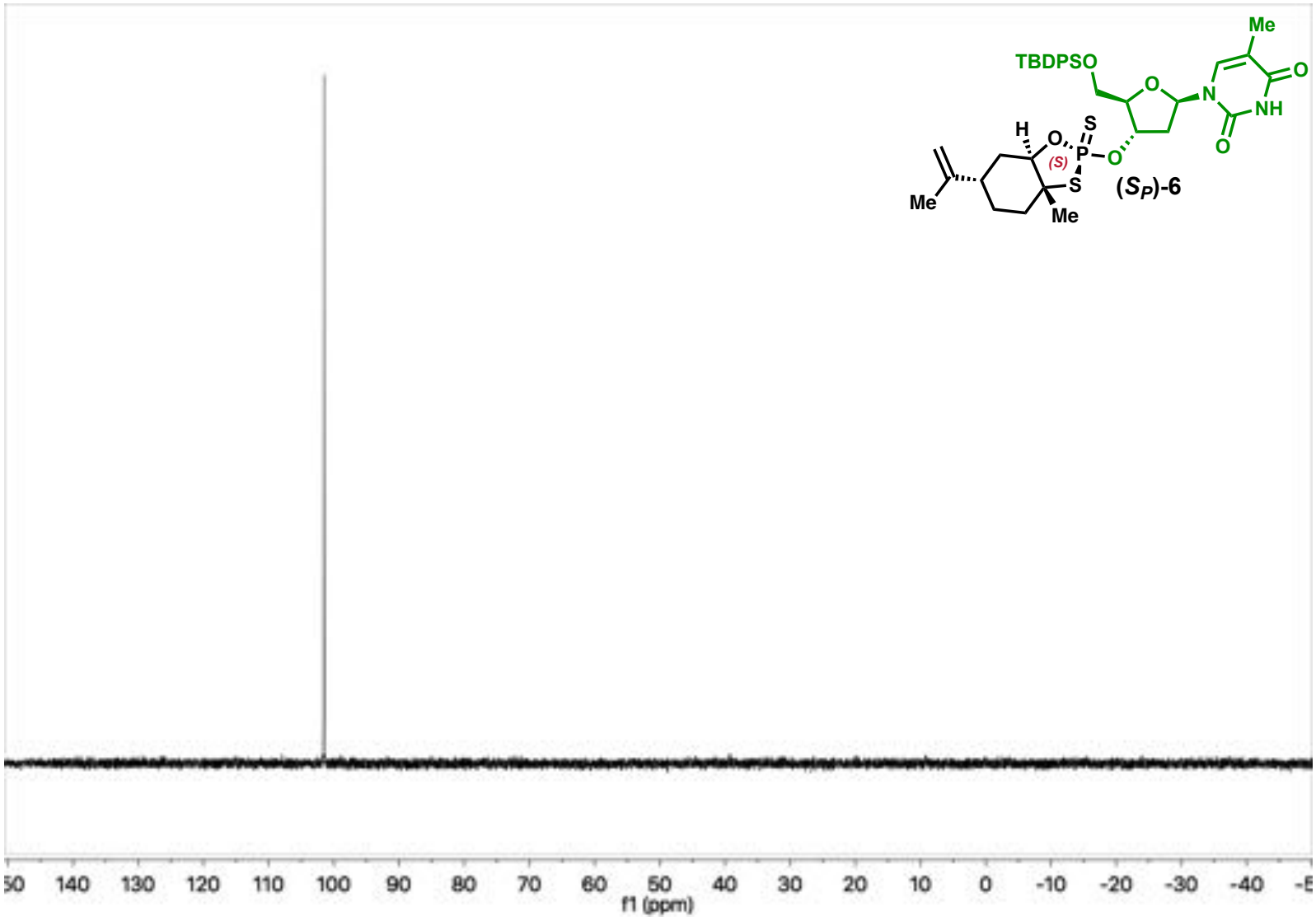
Compound (S_P)-6 ^{1}H NMR



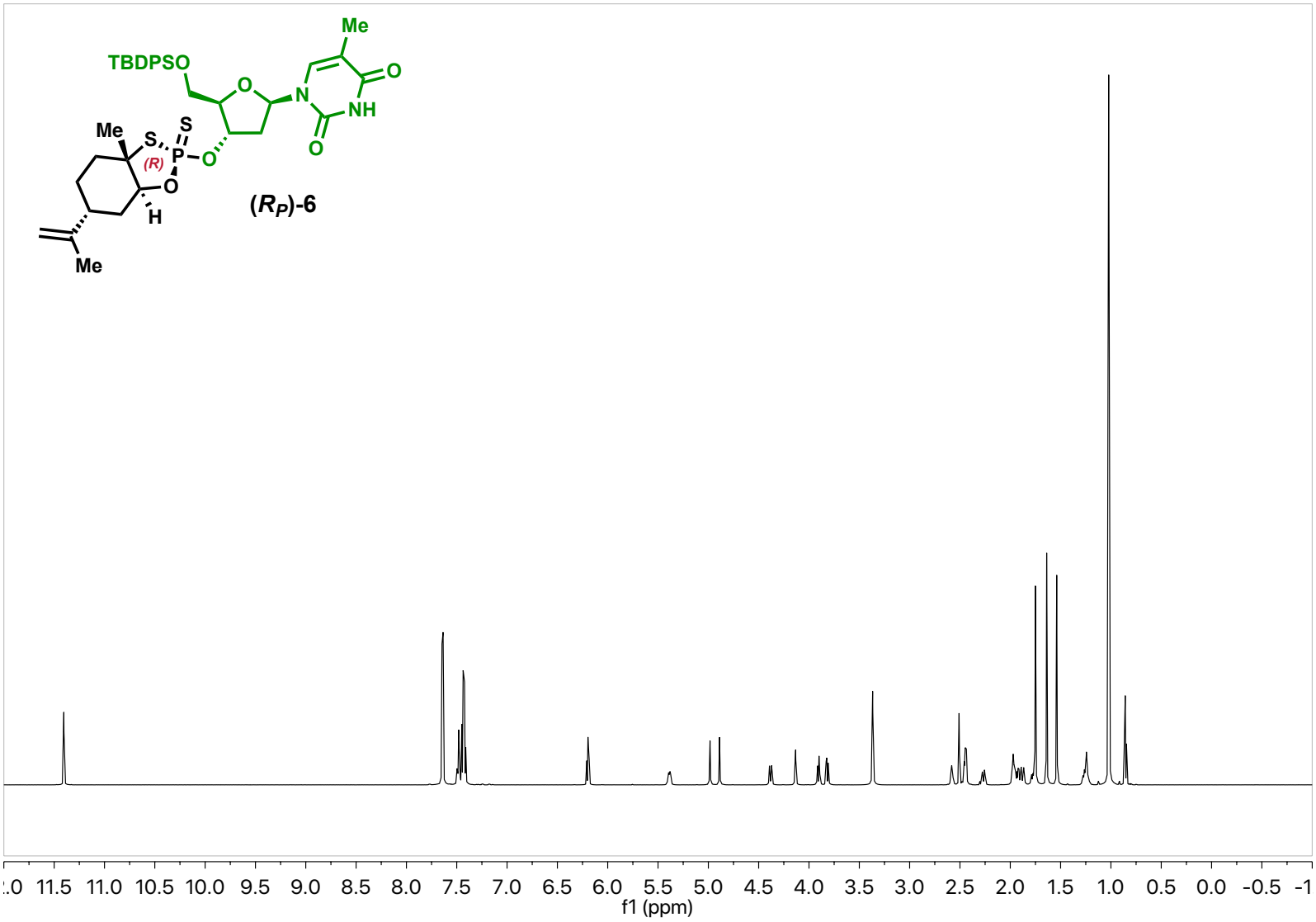
Compound (S_P)-6 ^{13}C NMR



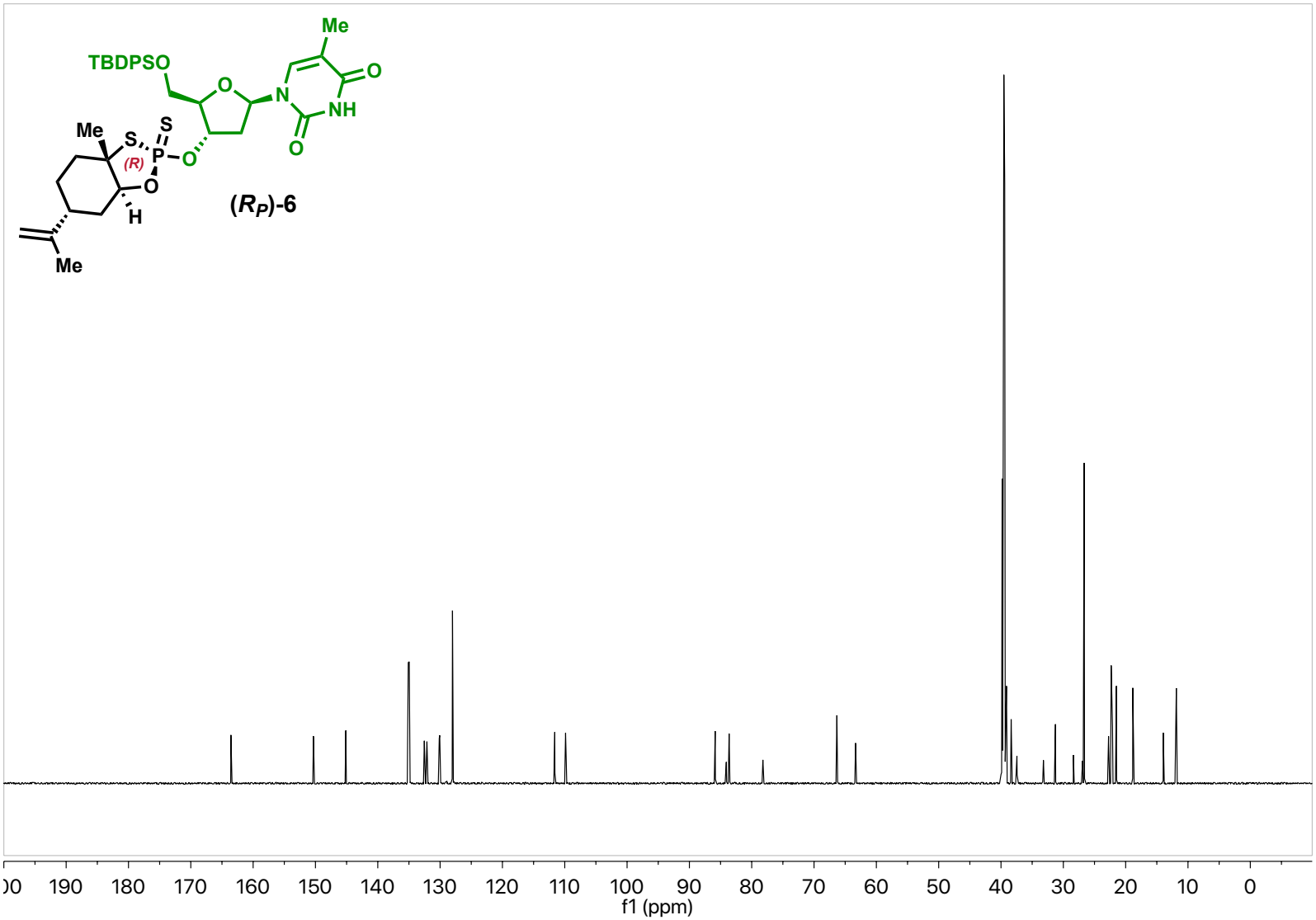
Compound (S_P)-6 ^{31}P NMR



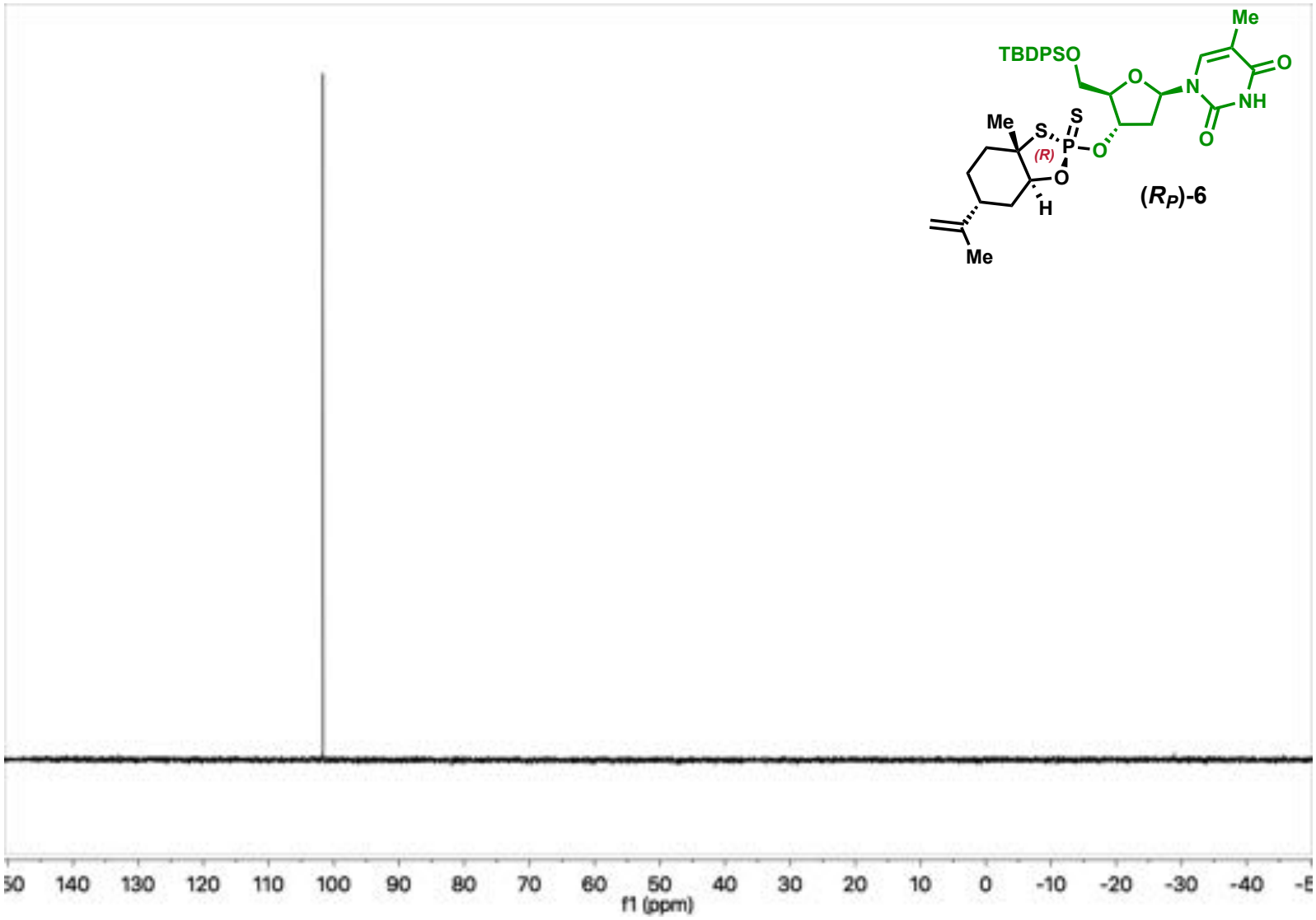
Compound (R_P)-6 ^{1}H NMR



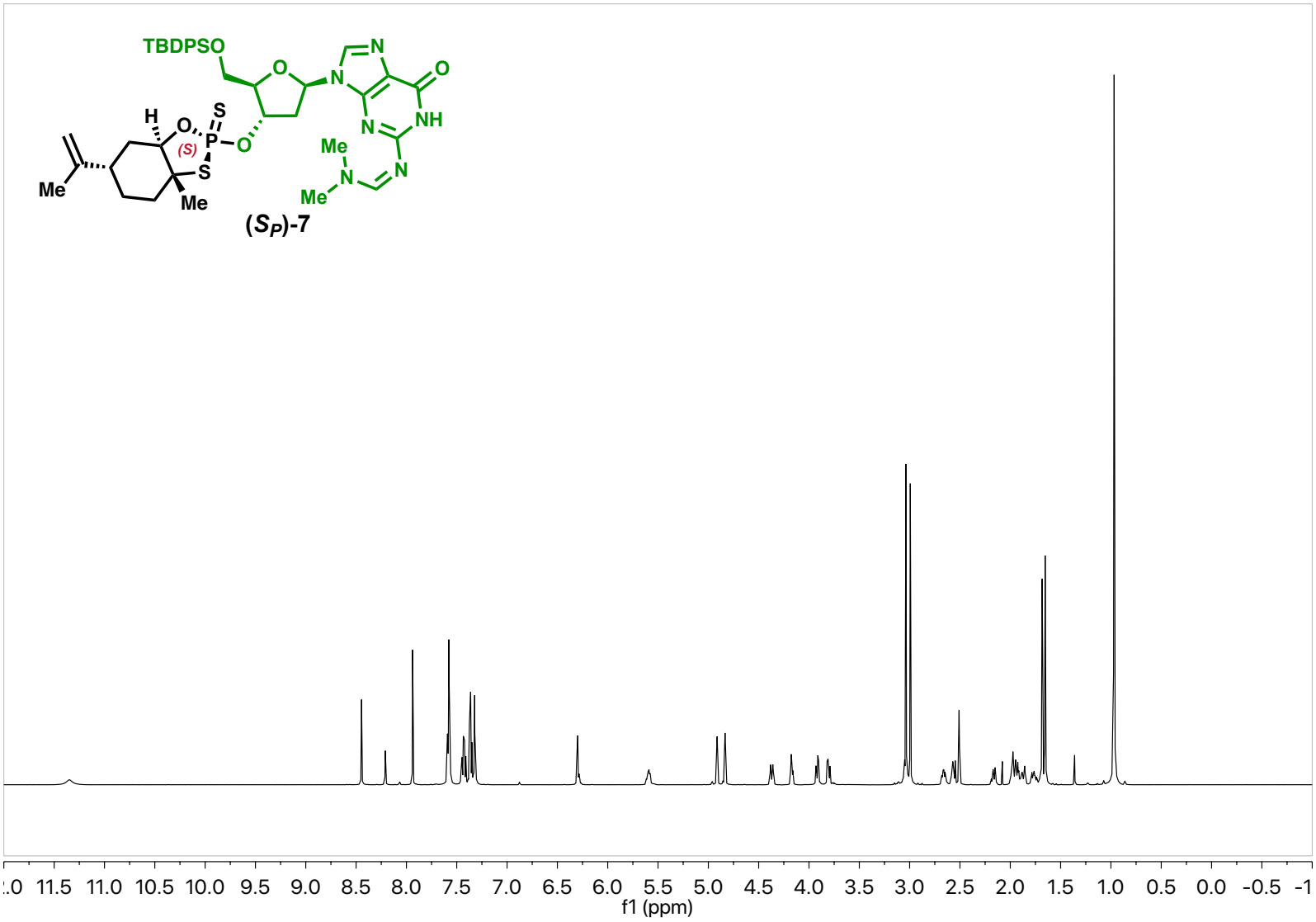
Compound (R_P)-6 ^{13}C NMR



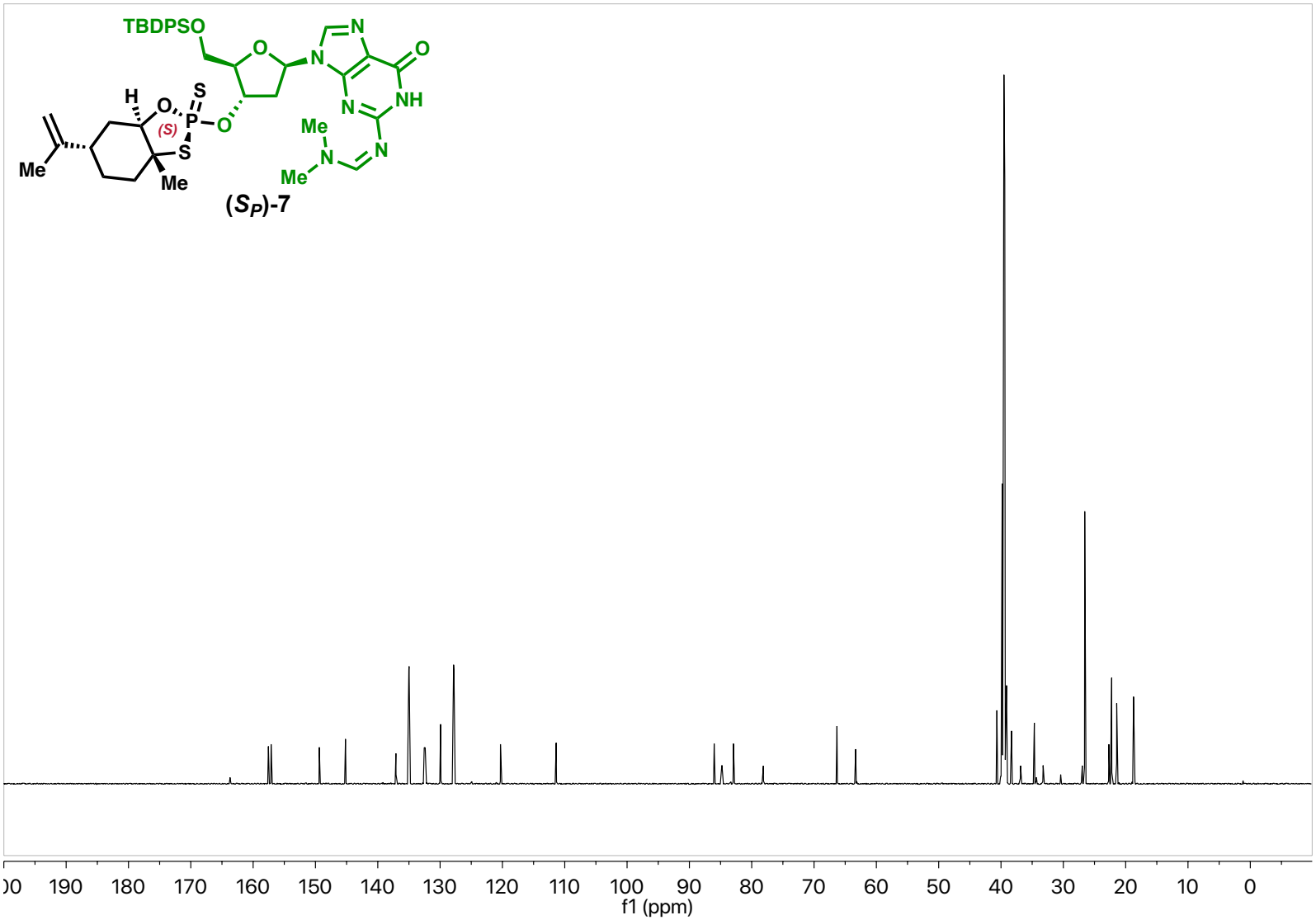
Compound (R_P)-6 ^{31}P NMR



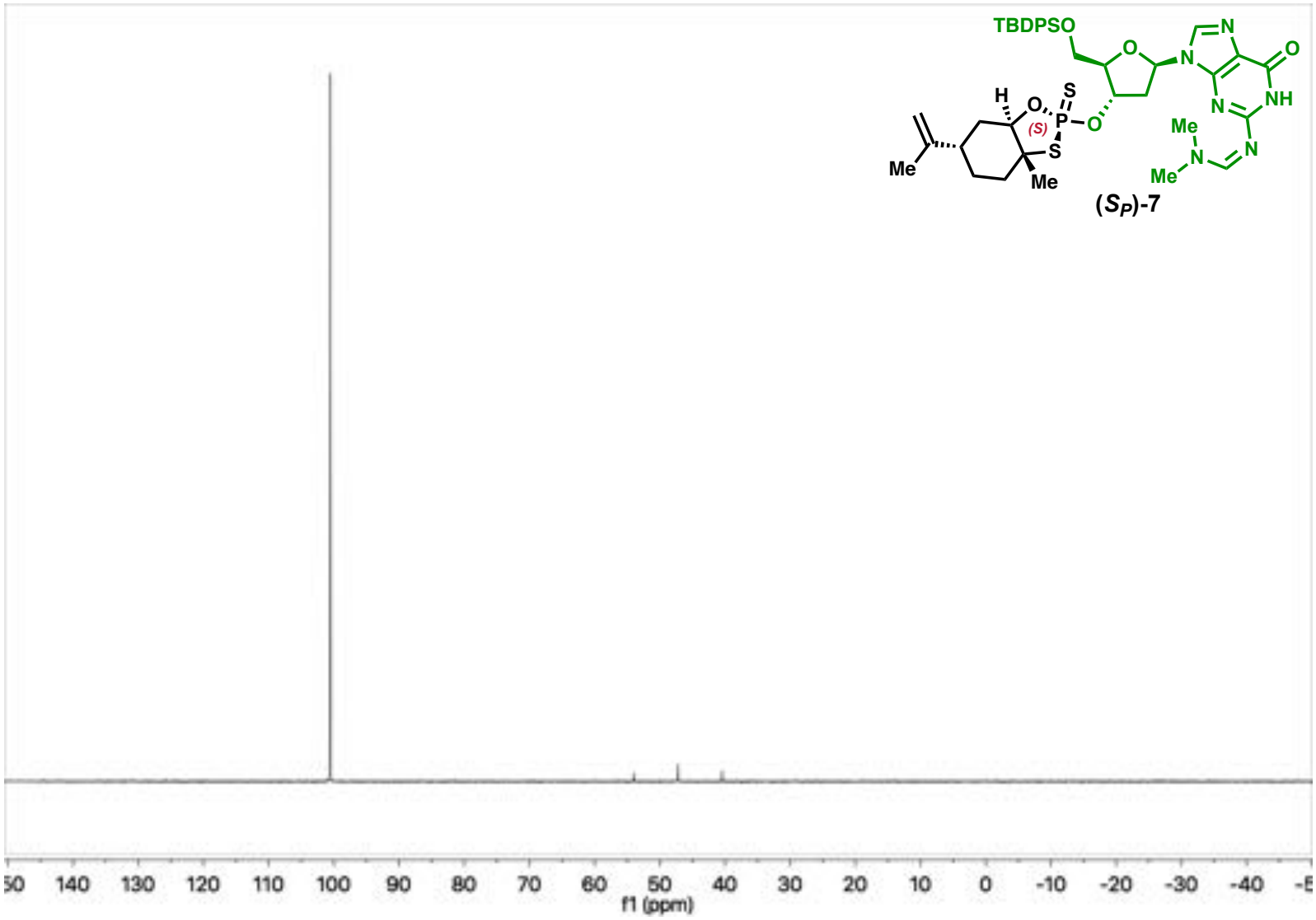
Compound (S_P)-7 ^{1}H NMR



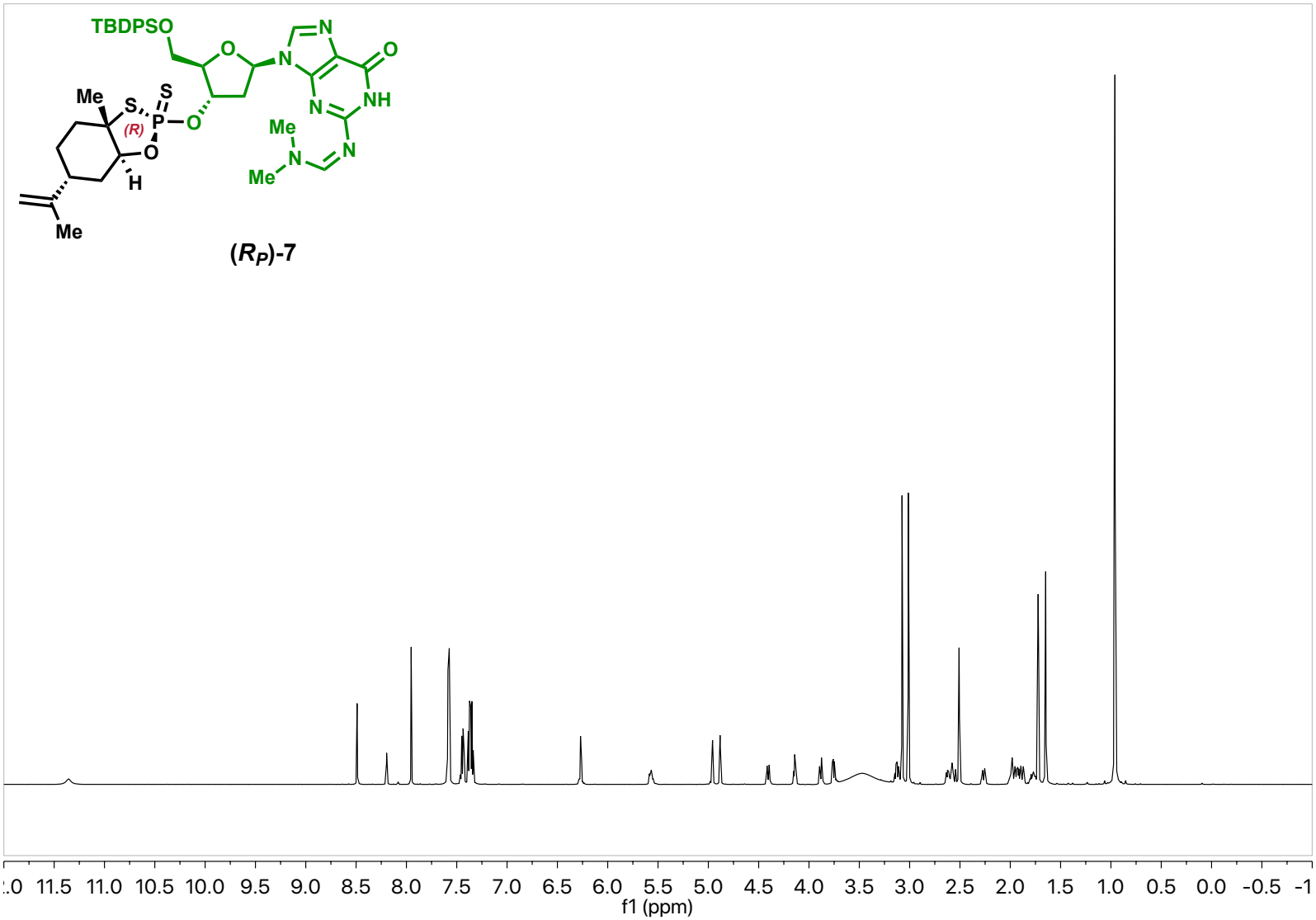
Compound (S_P)-7 ^{13}C NMR



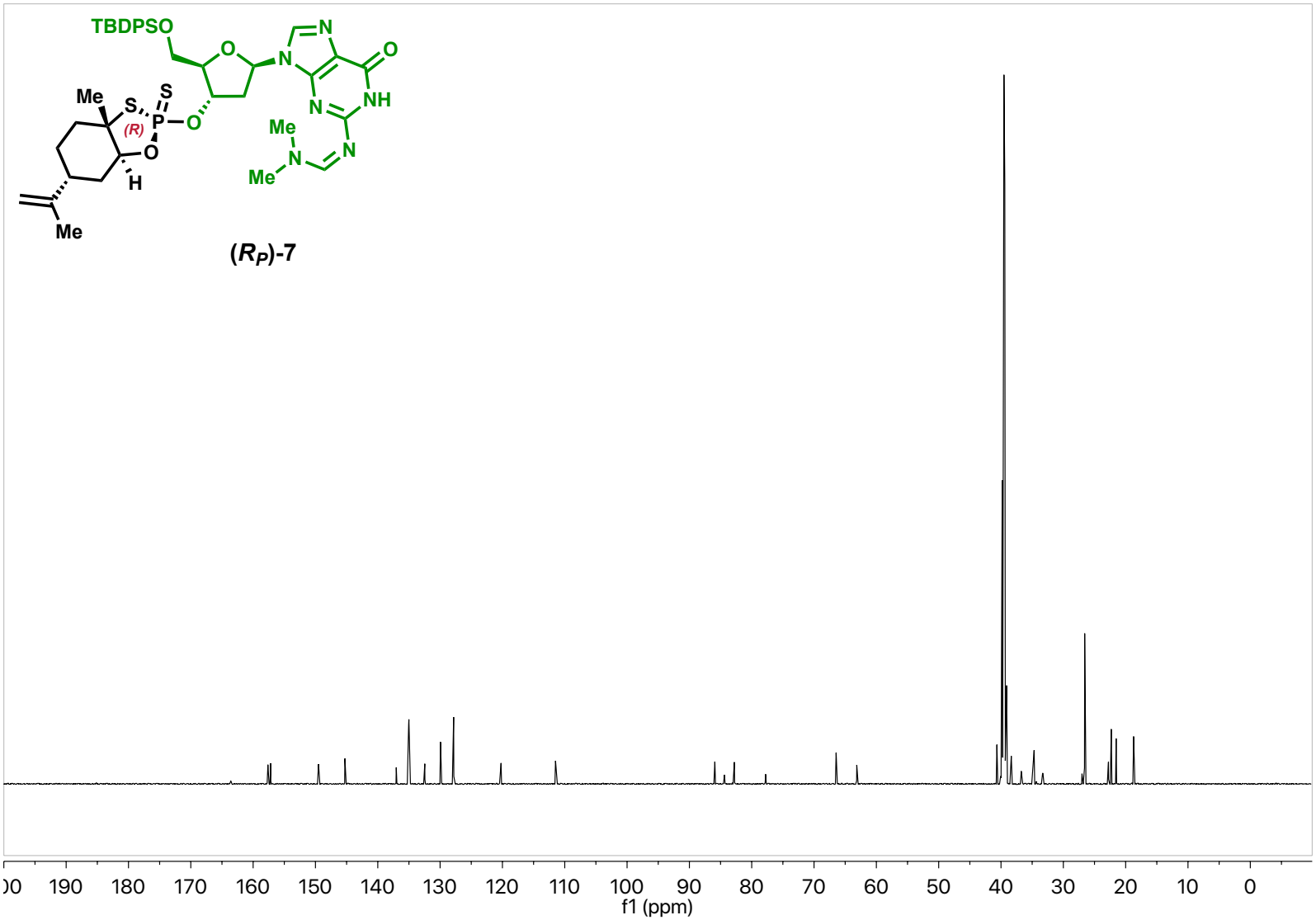
Compound (S_P)-7 ^{31}P NMR



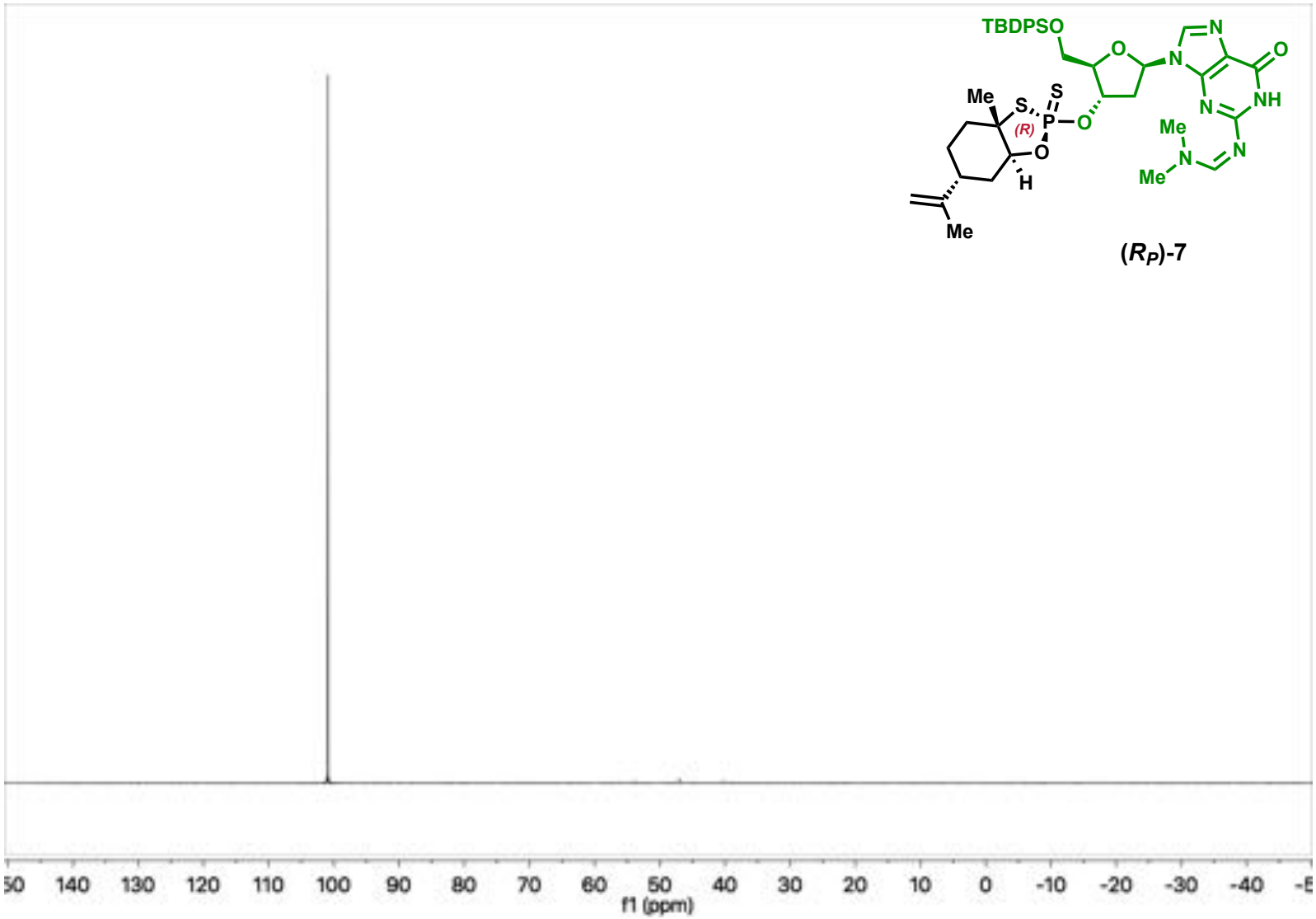
Compound (R_P)-7 ^{1}H NMR



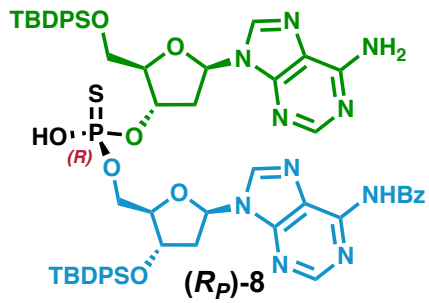
Compound (R_P)-7 ^{13}C NMR



Compound (R_P)-7 ^{31}P NMR

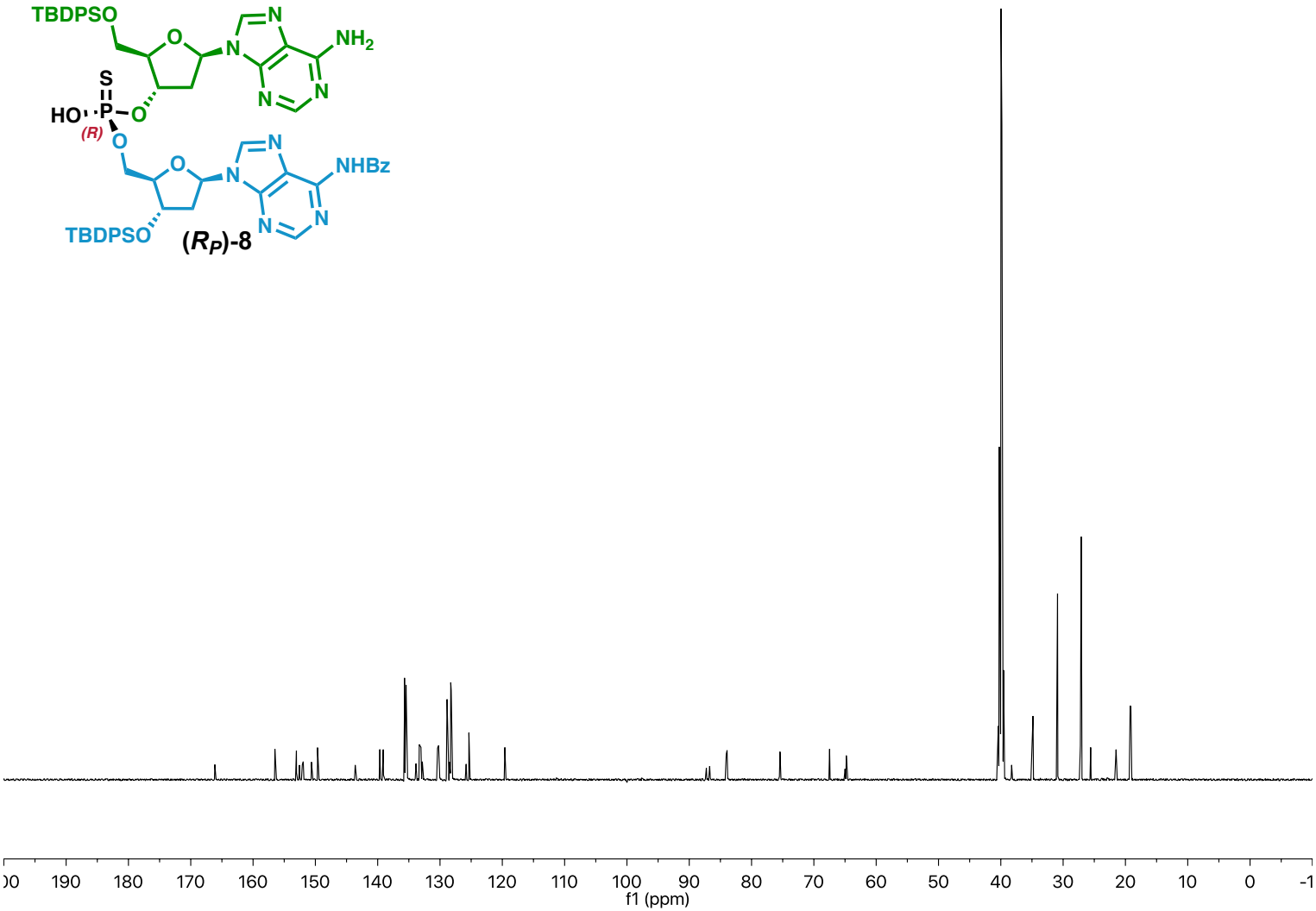


Compound (R_P)-8 ^{1}H NMR

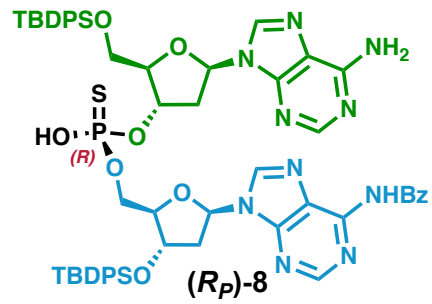


(R_P)-8

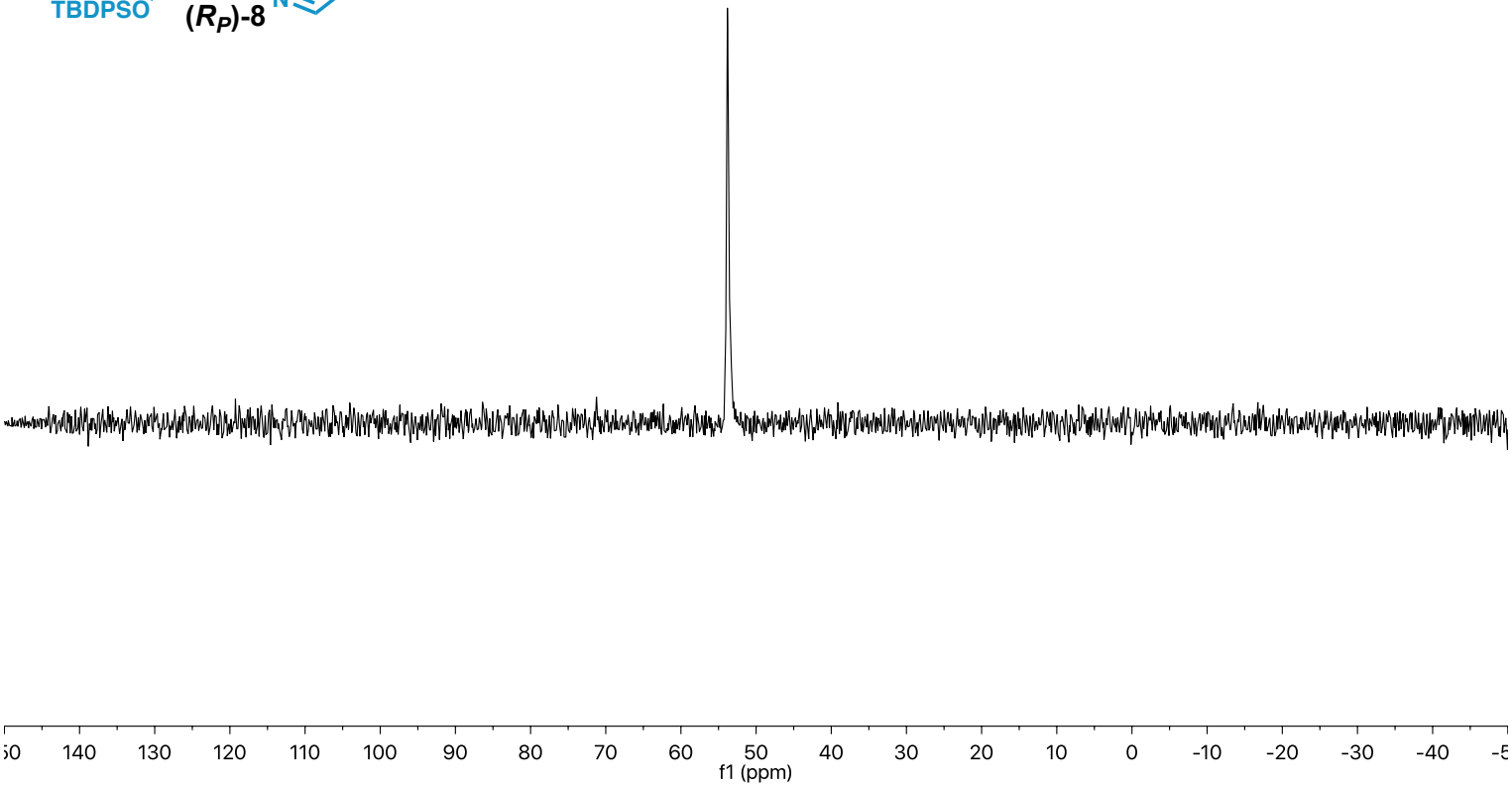
Compound (R_P)-8 ^{13}C NMR



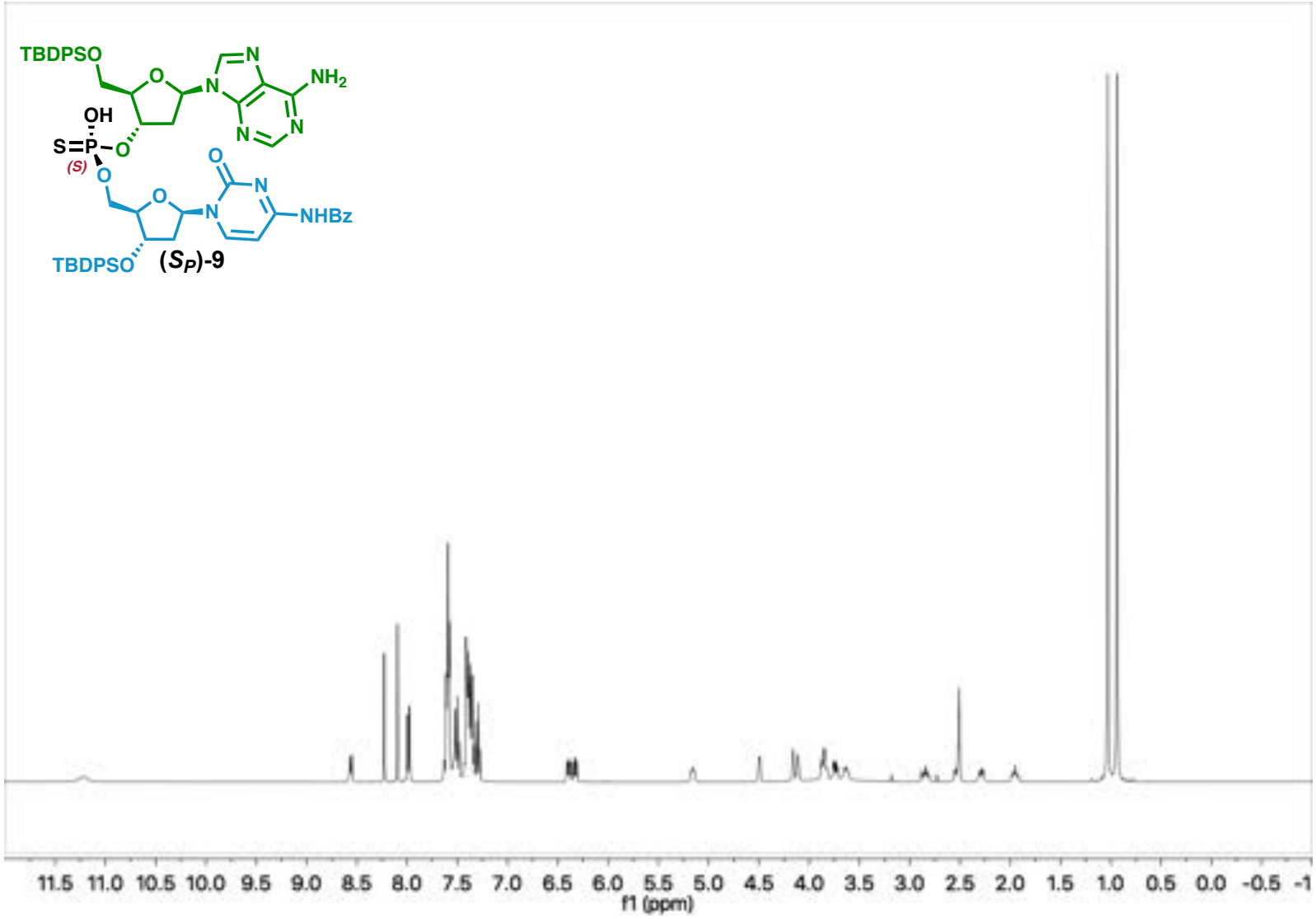
Compound (R_P)-8 ^{31}P NMR



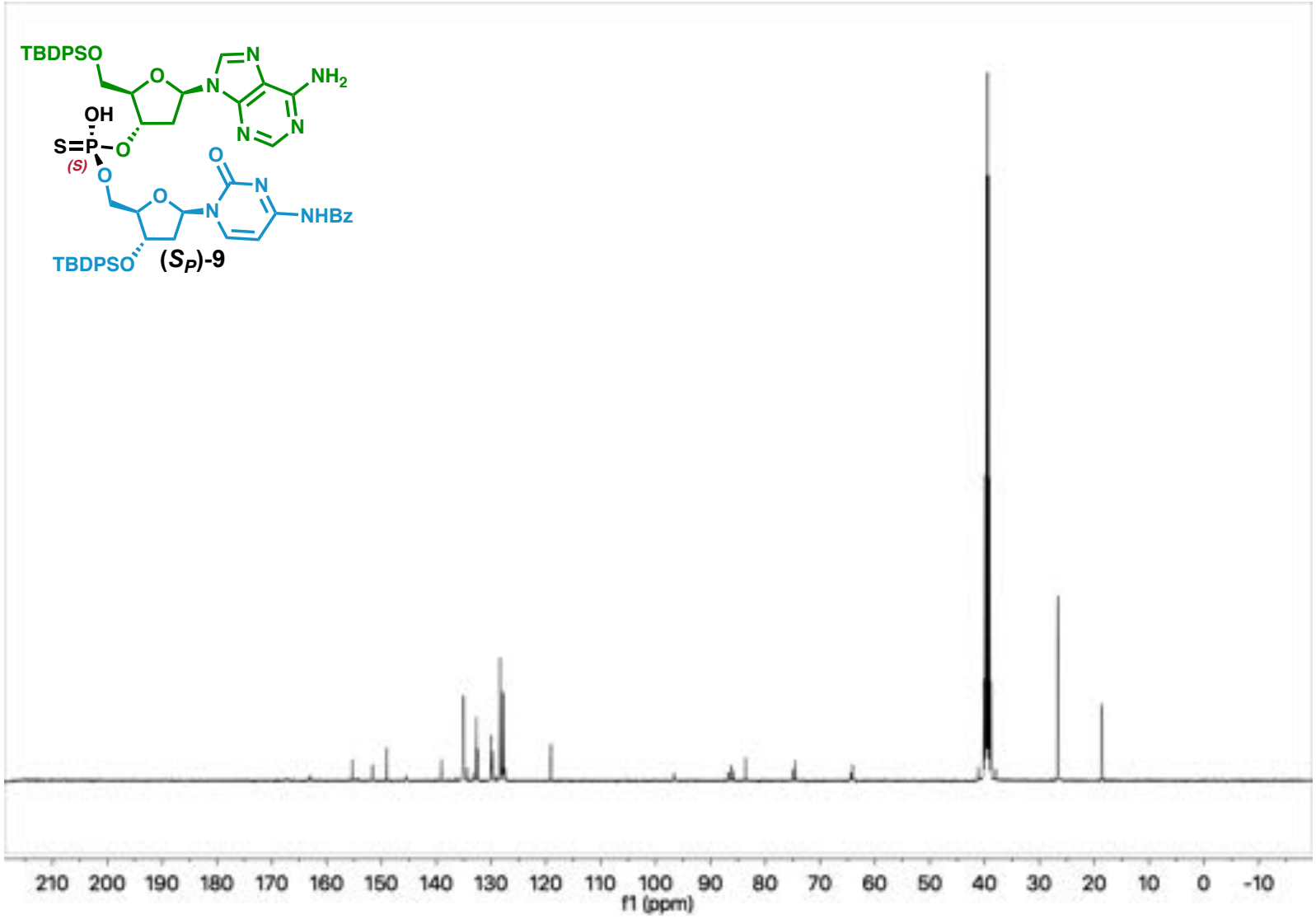
(R_P)-8



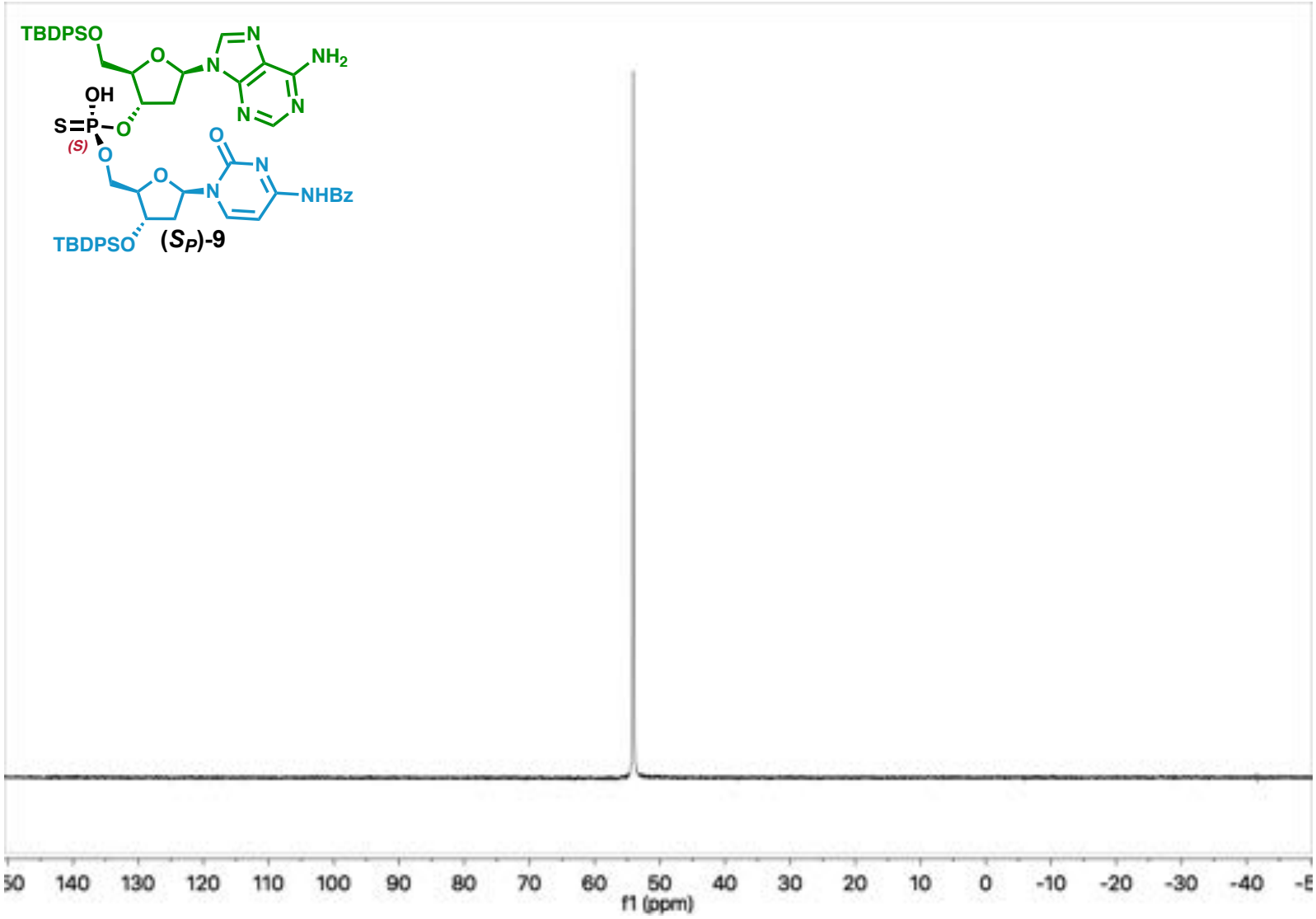
Compound (S_P)-9 ^{1}H NMR



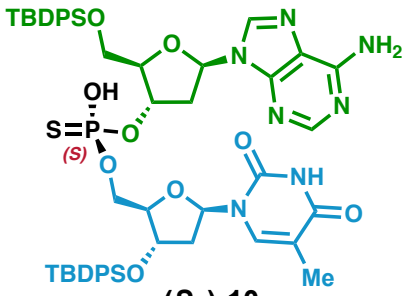
Compound (S_P)-9 ^{13}C NMR



Compound (S_P)-9 ^{31}P NMR



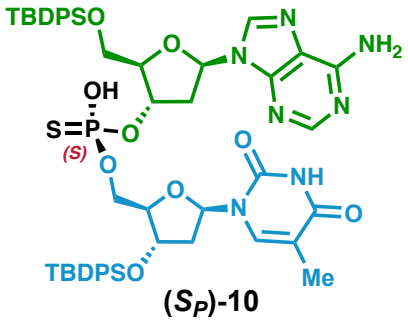
Compound (S_P)-10 ^{1}H NMR


TBDPSO
O
N
N
N
N
NH2
OH
S=P–O
(S)
O
O
NH
O
N
O
TBDPSO
Me

(S_P)-10

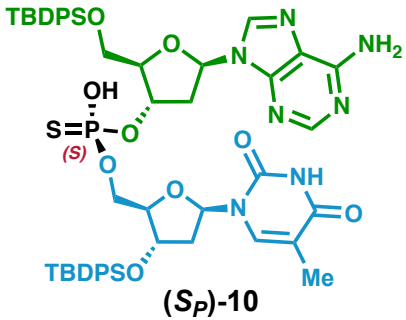
.0 11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5 -1
f1 (ppm)

Compound (S_P)-10 ^{13}C NMR

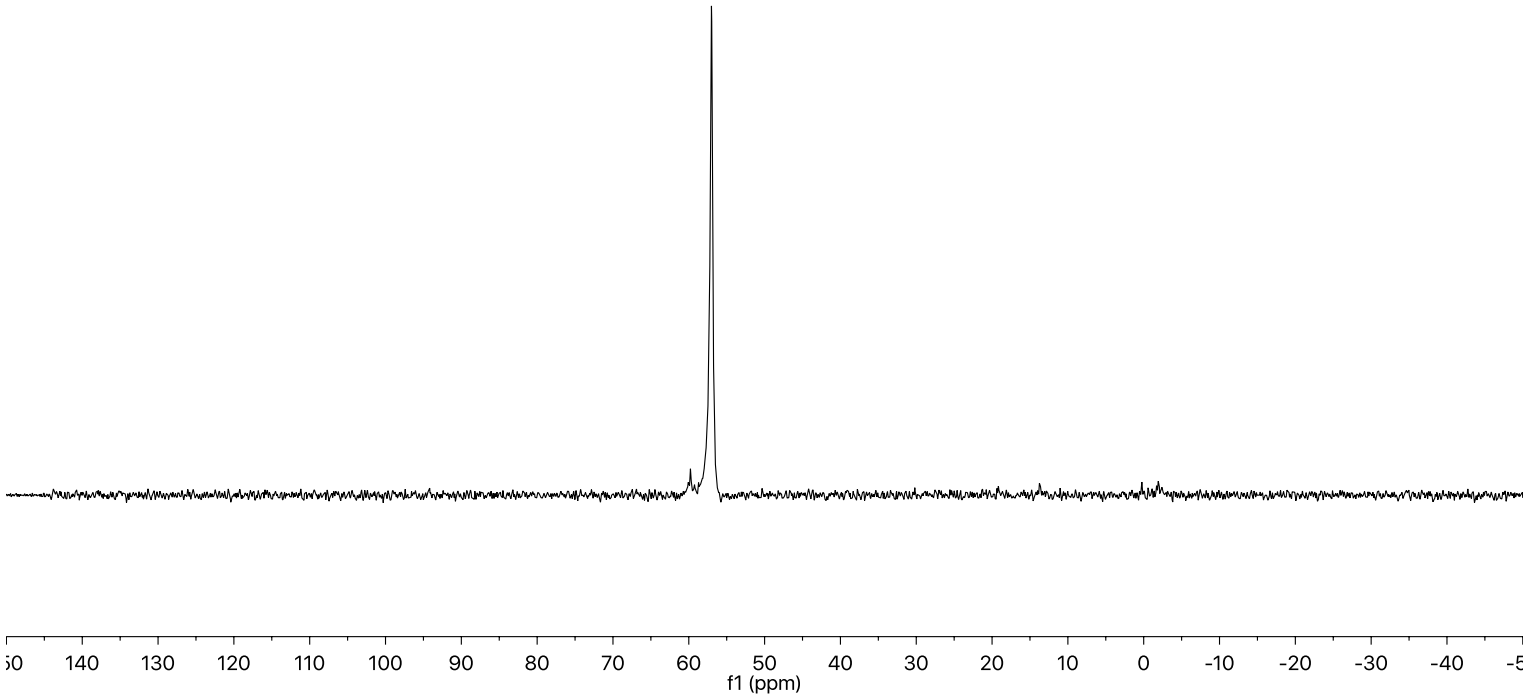


(S_P)-10

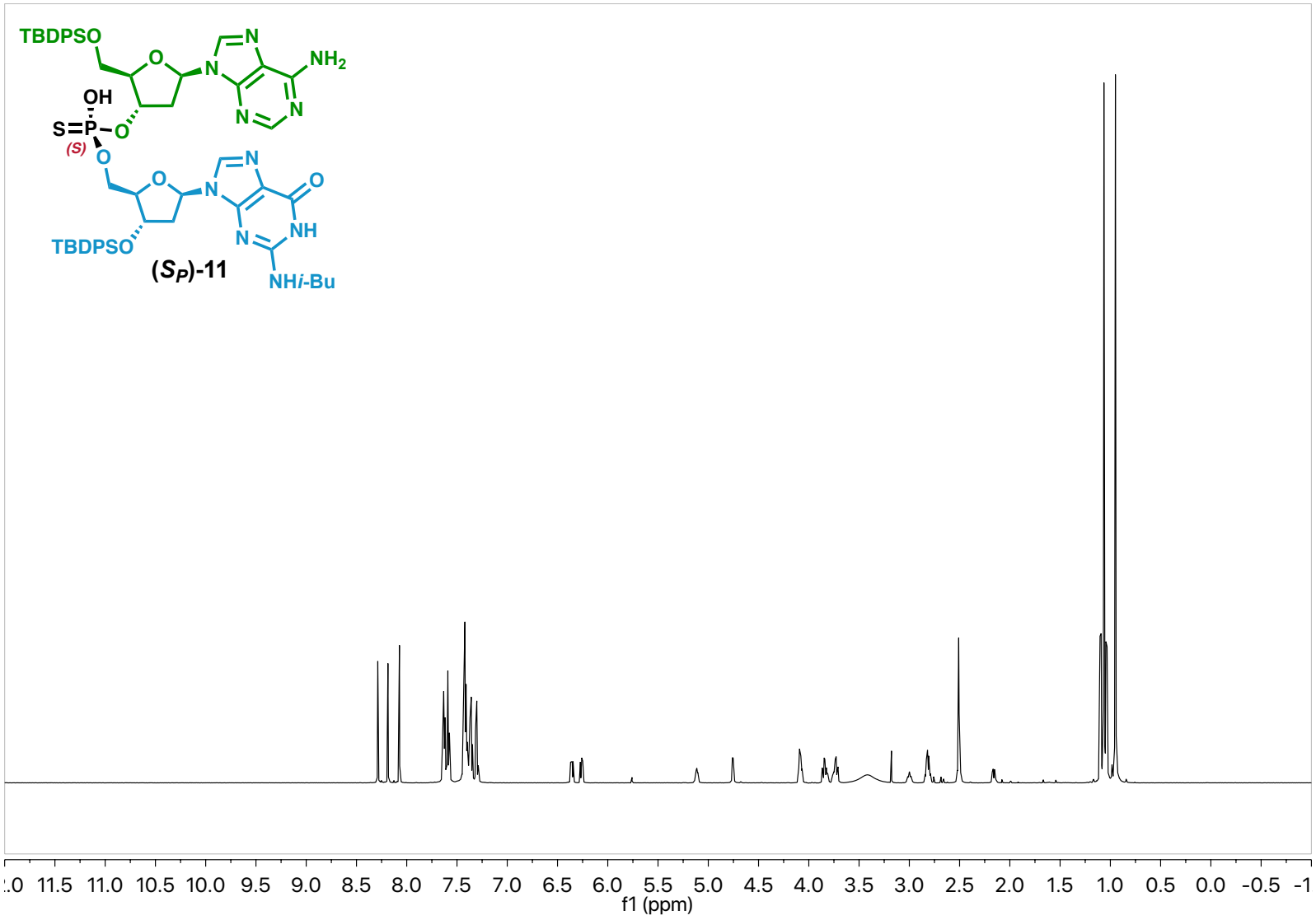
Compound (S_P)-10 ^{31}P NMR



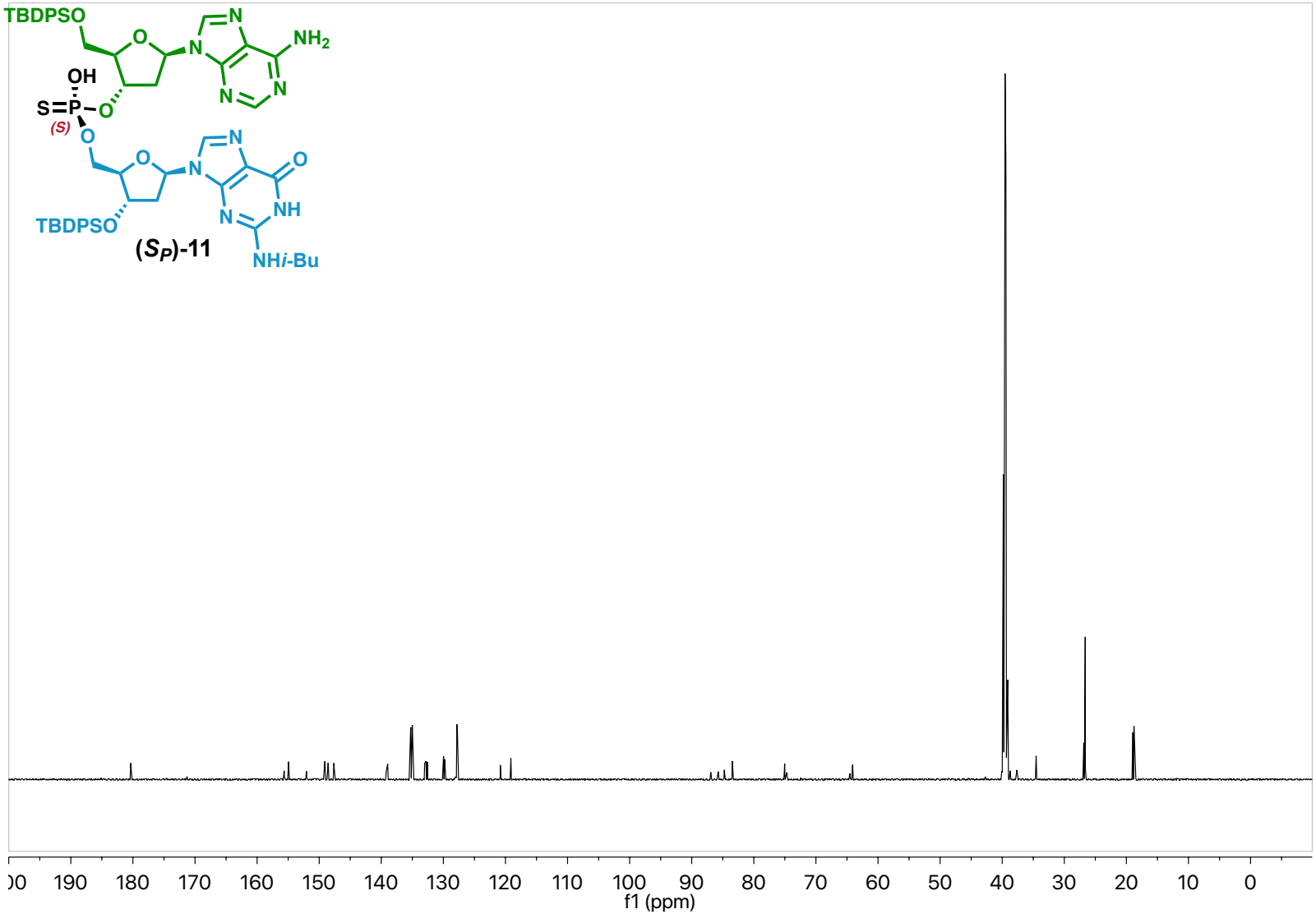
(S_P)-10



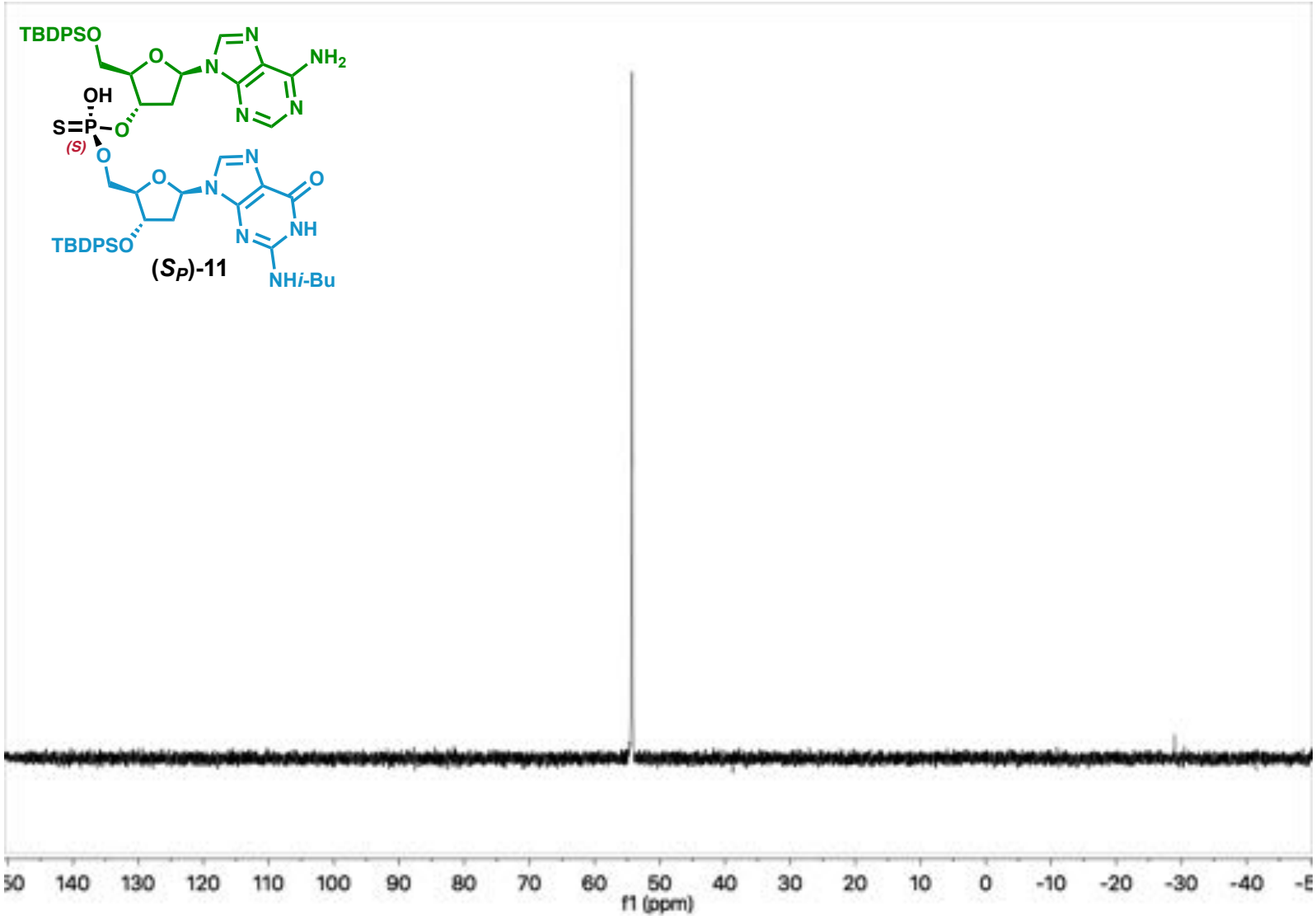
Compound (S_P)-11 ^{1}H NMR



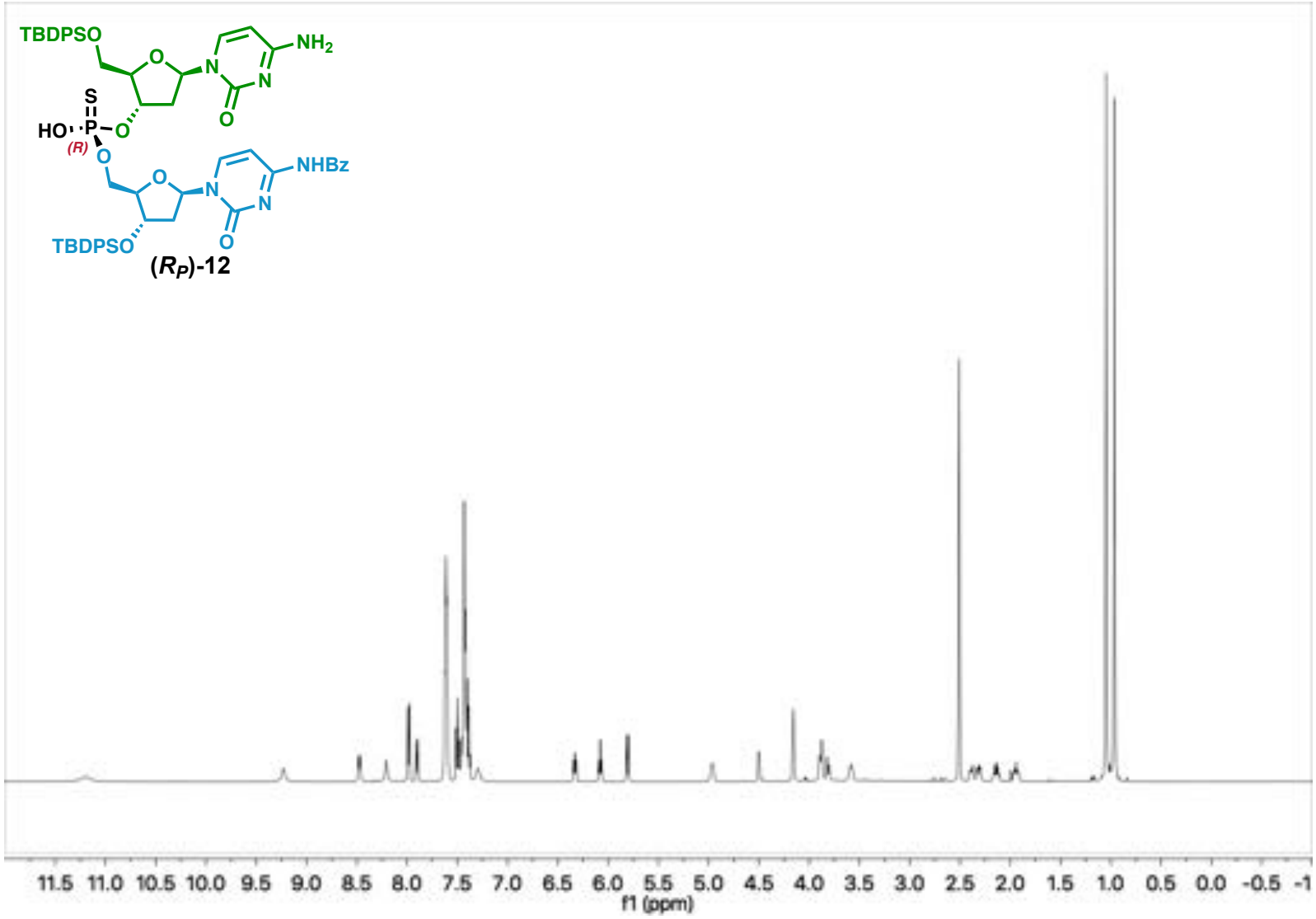
Compound (S_P)-11 ^{13}C NMR



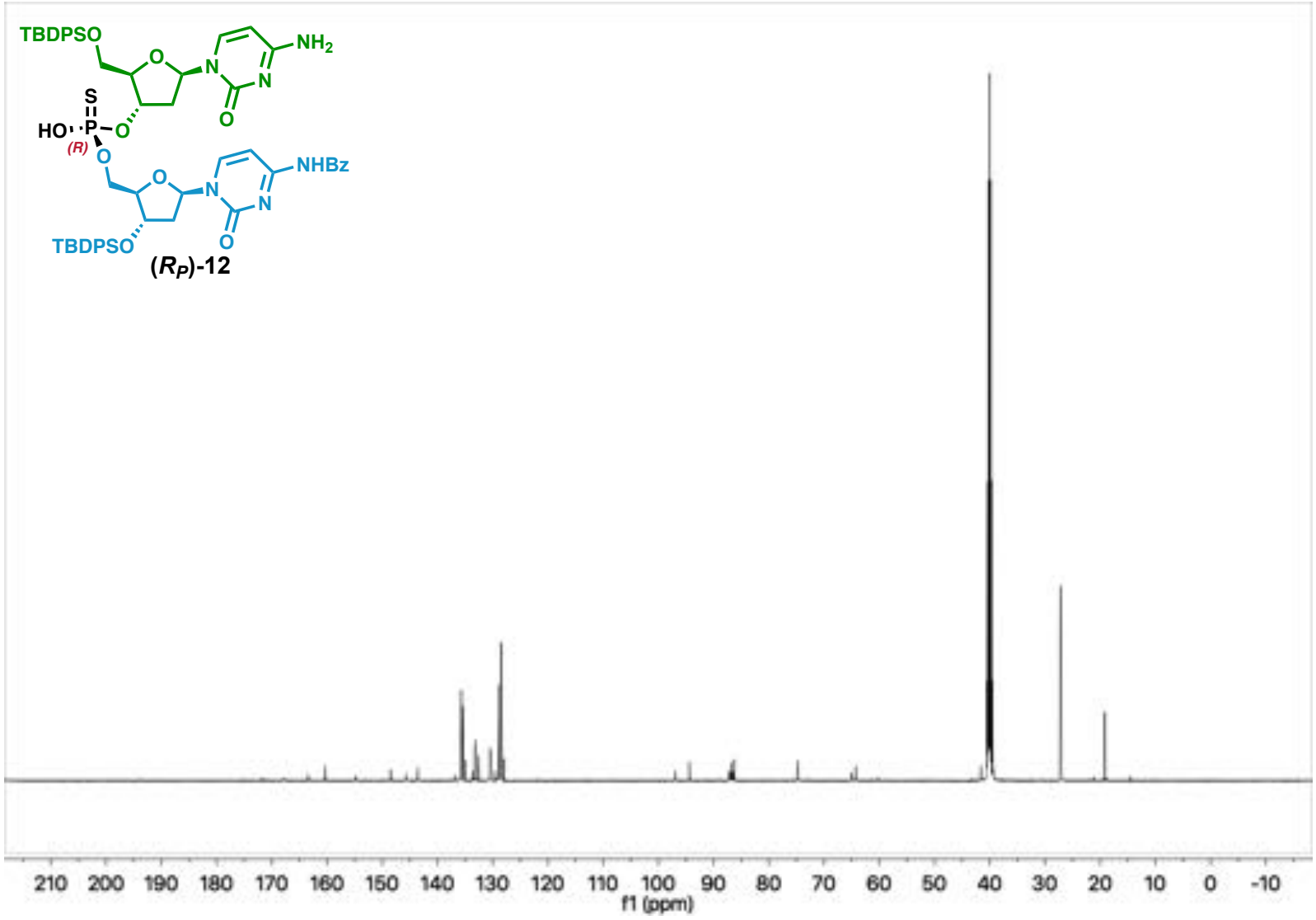
Compound (S_P)-11 ^{31}P NMR



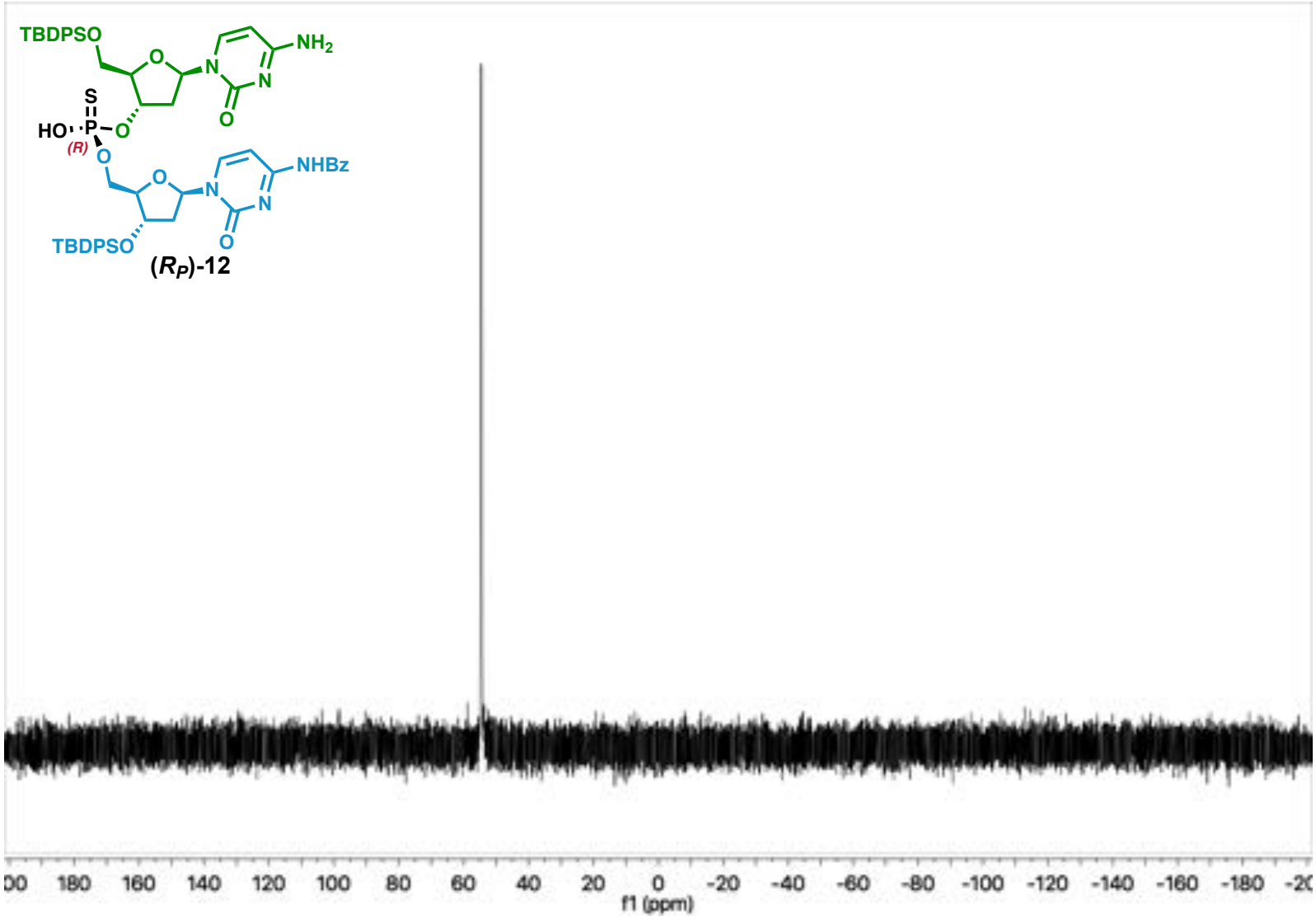
Compound (R_P)-12 ^{1}H NMR



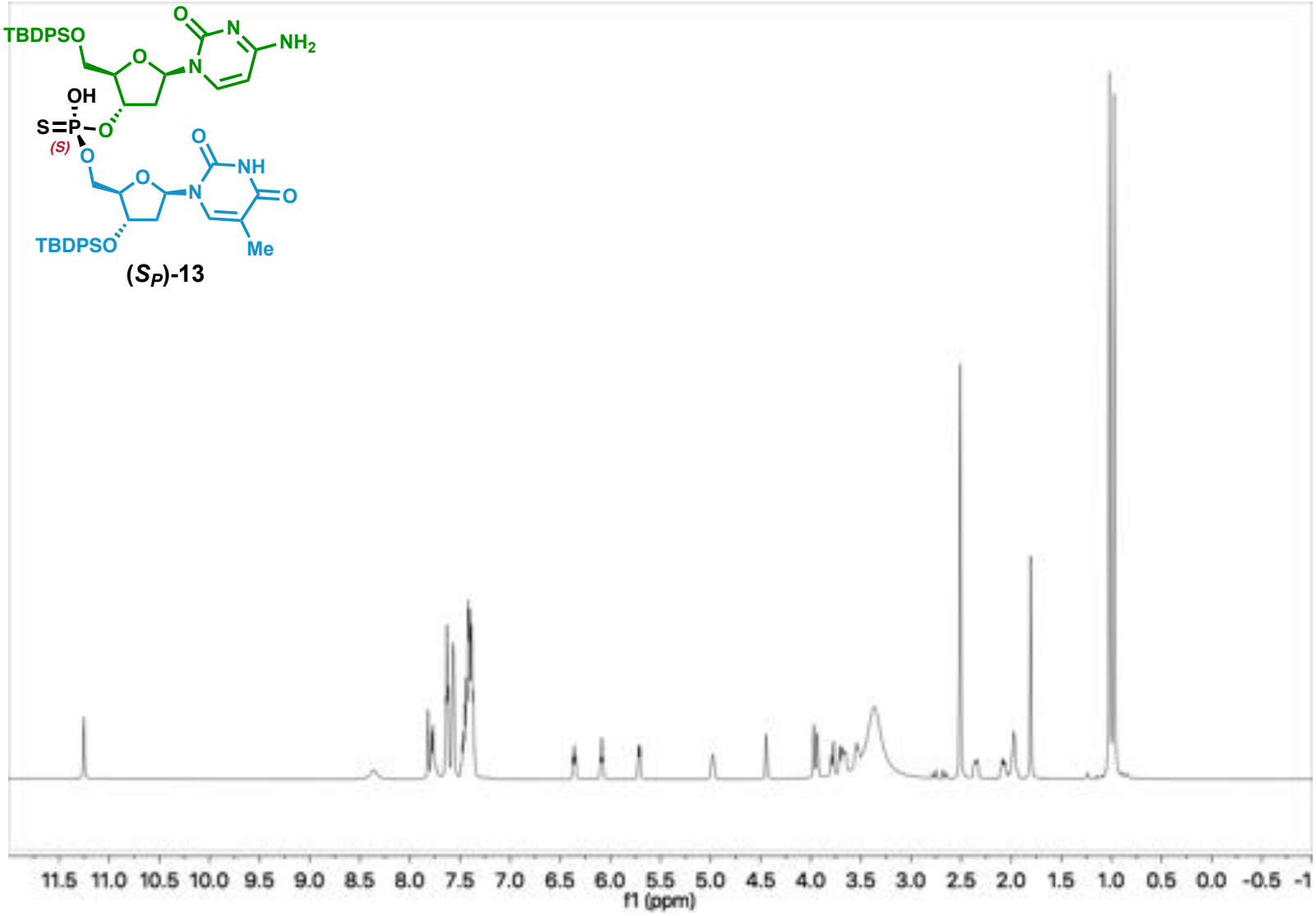
Compound (R_P)-12 ^{13}C NMR



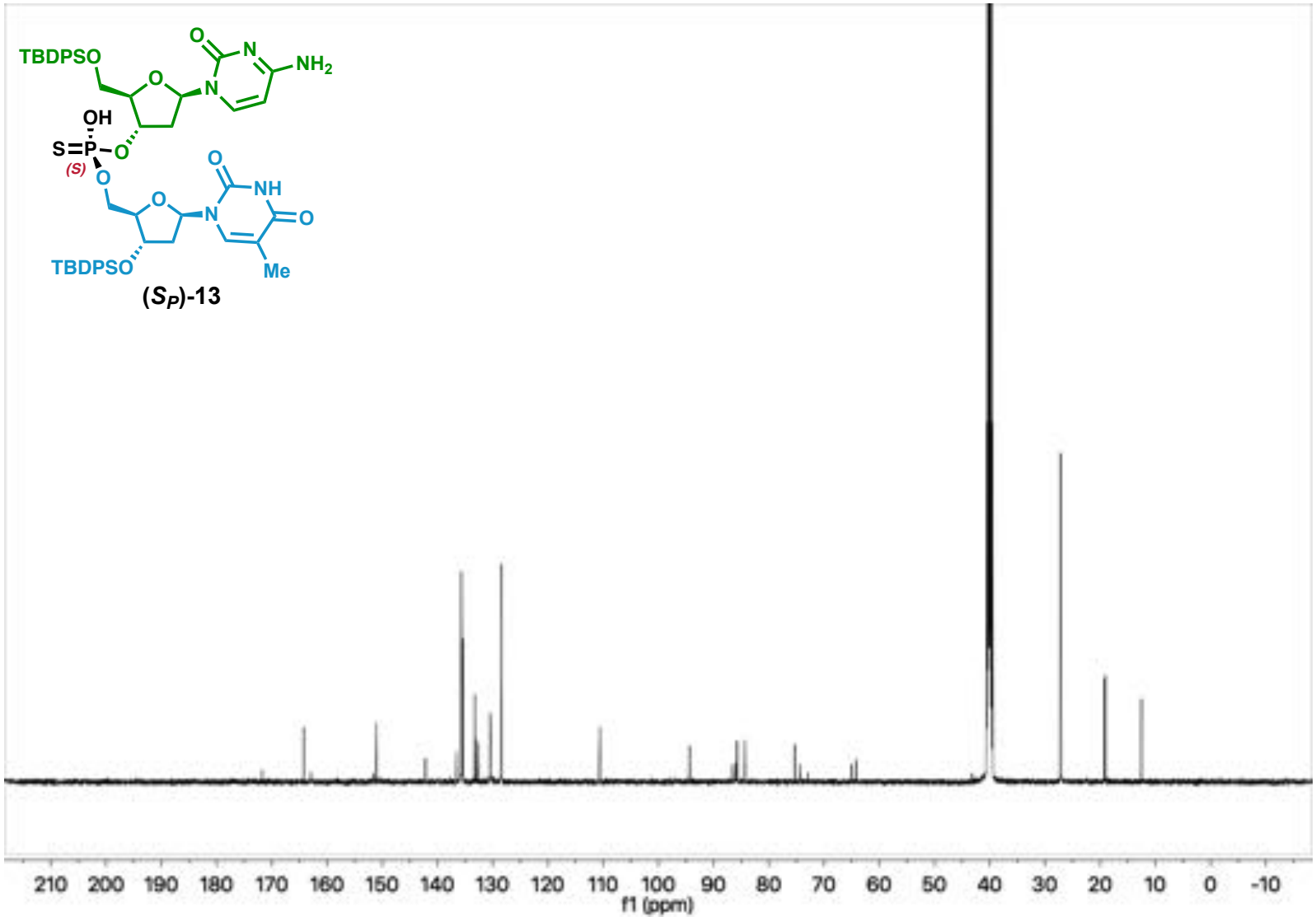
Compound (R_P)-12 ^{31}P NMR



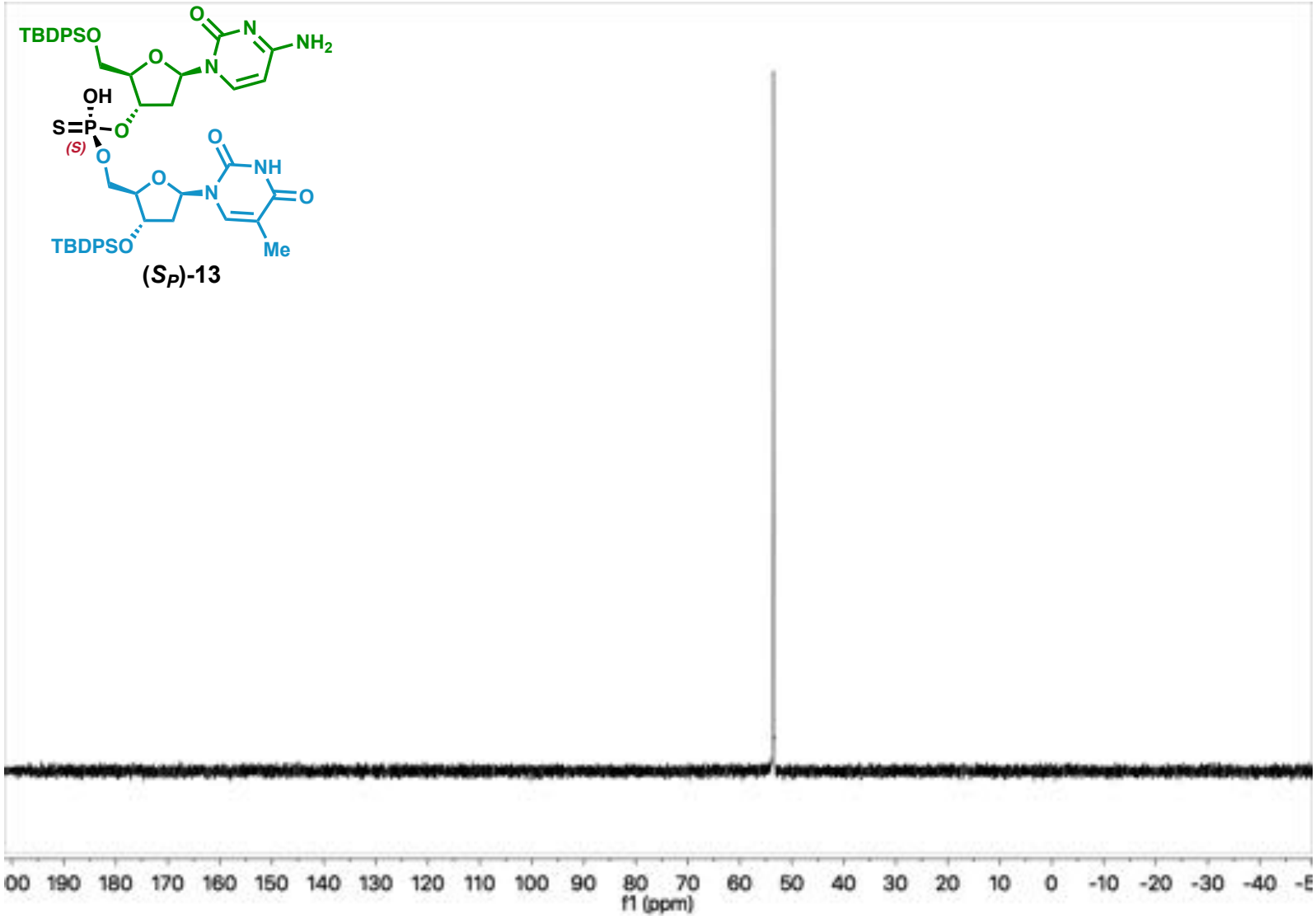
Compound (S_P)-13 ^{1}H NMR



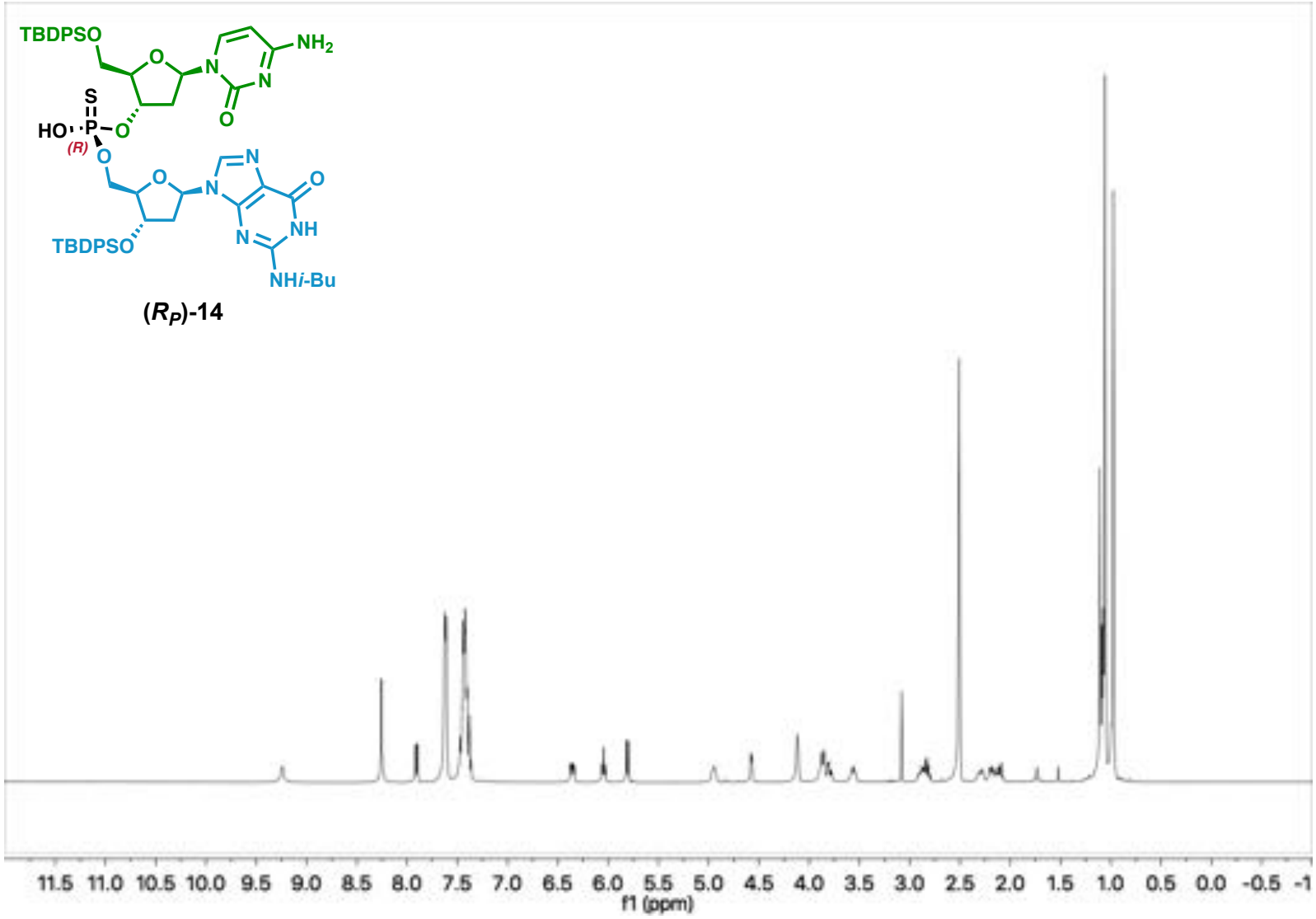
Compound (S_P)-13 ^{13}C NMR



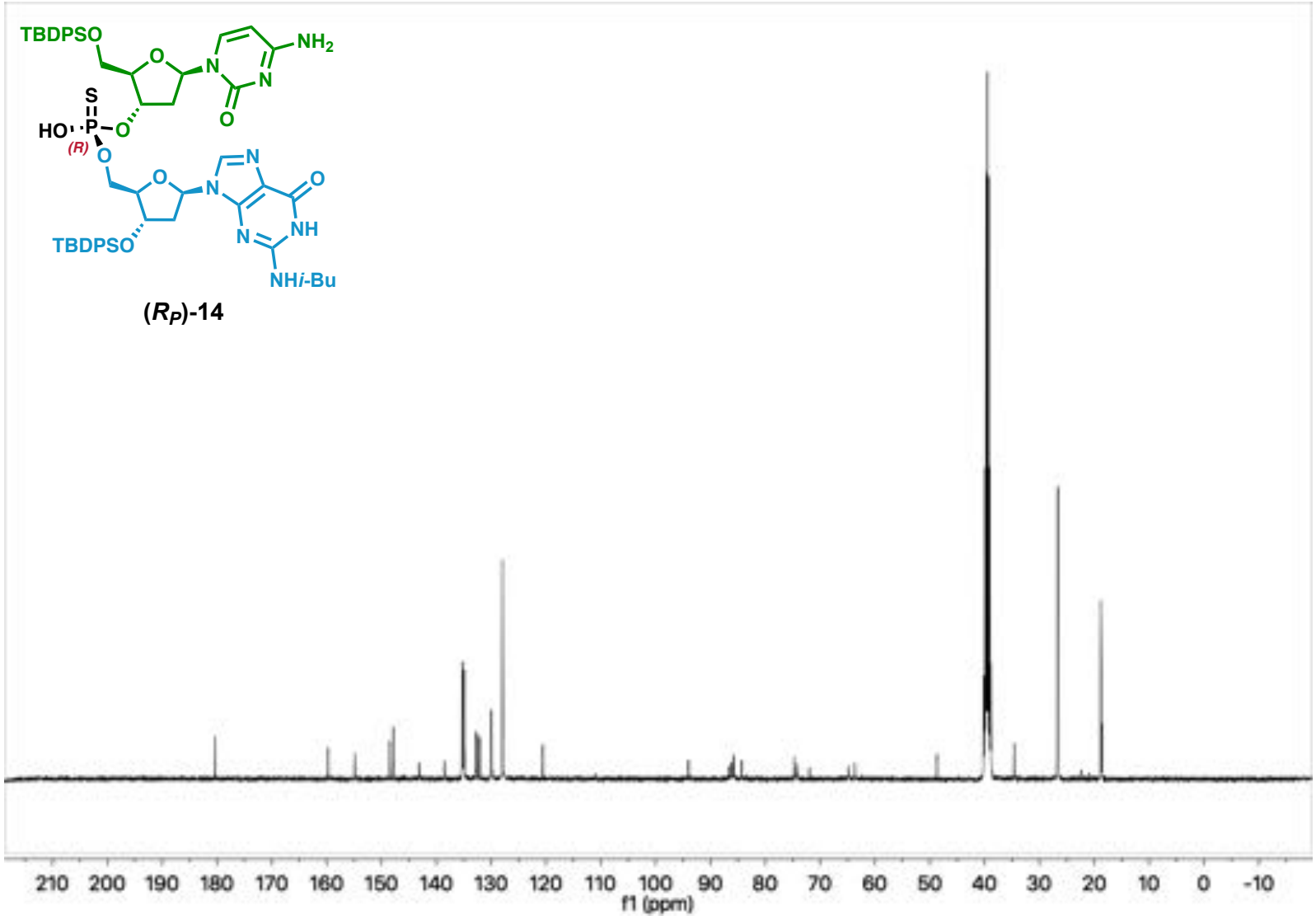
Compound (S_P)-13 ^{31}P NMR



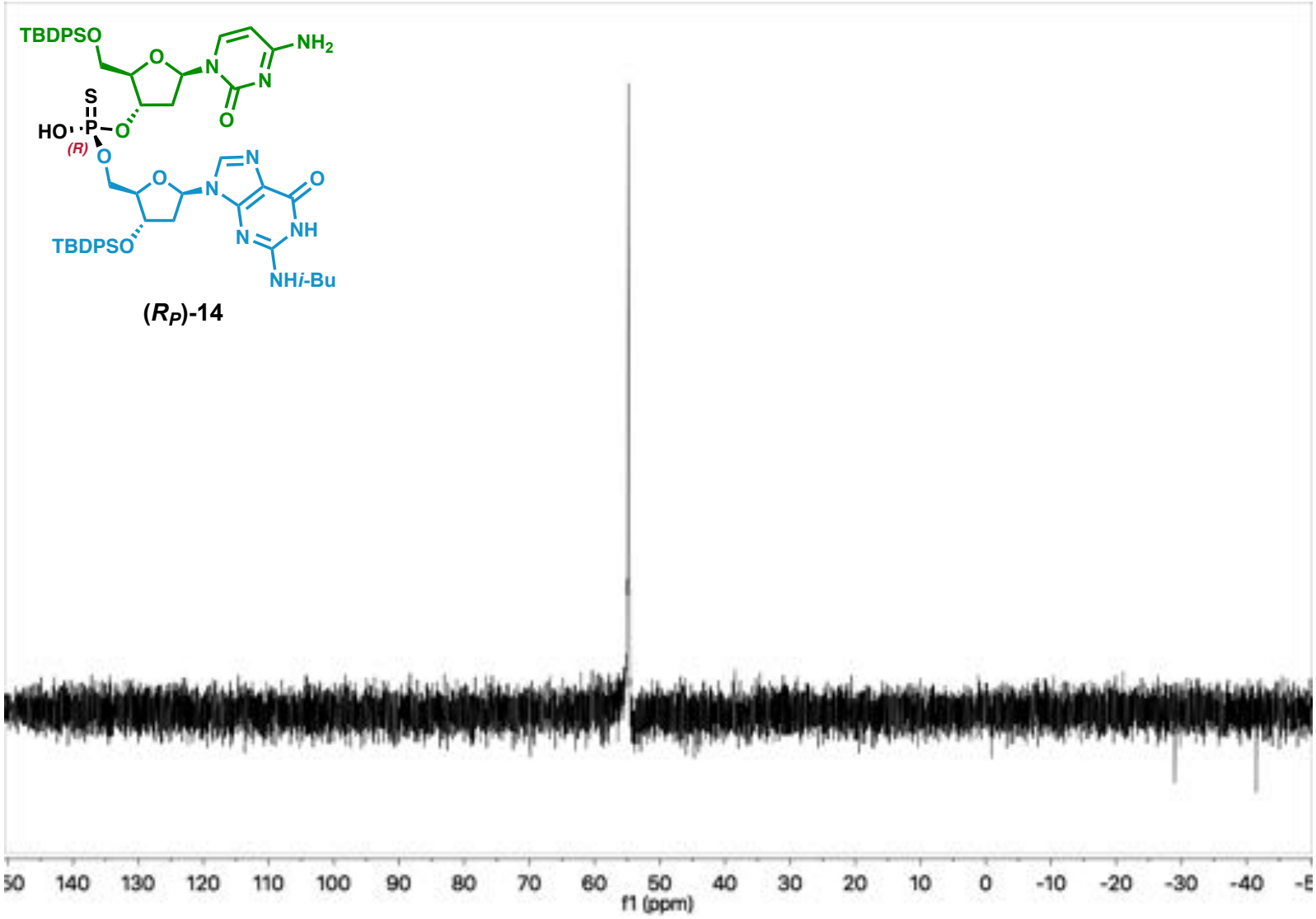
Compound (R_P)-14 ^{1}H NMR



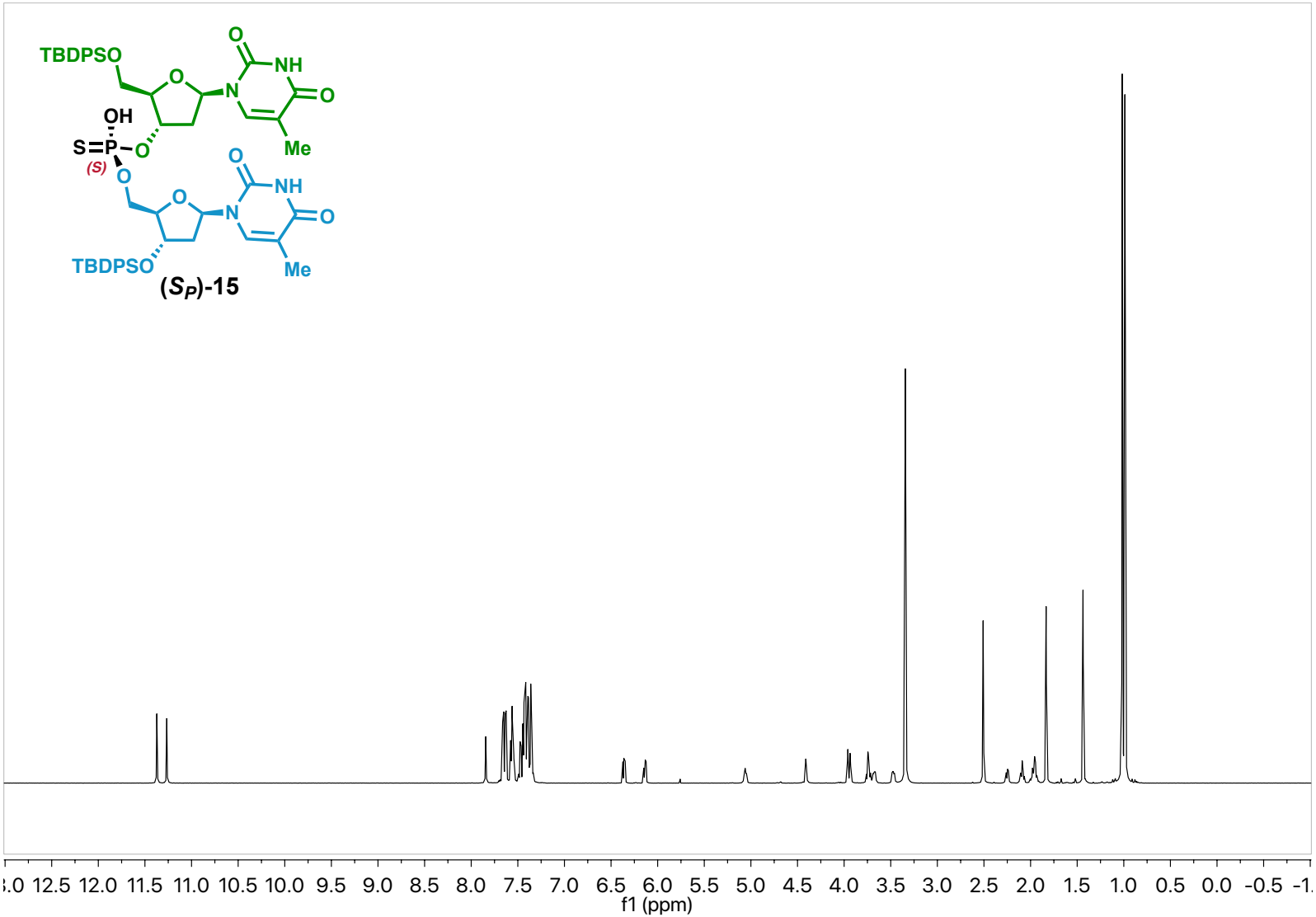
Compound (R_P)-14 ^{13}C NMR



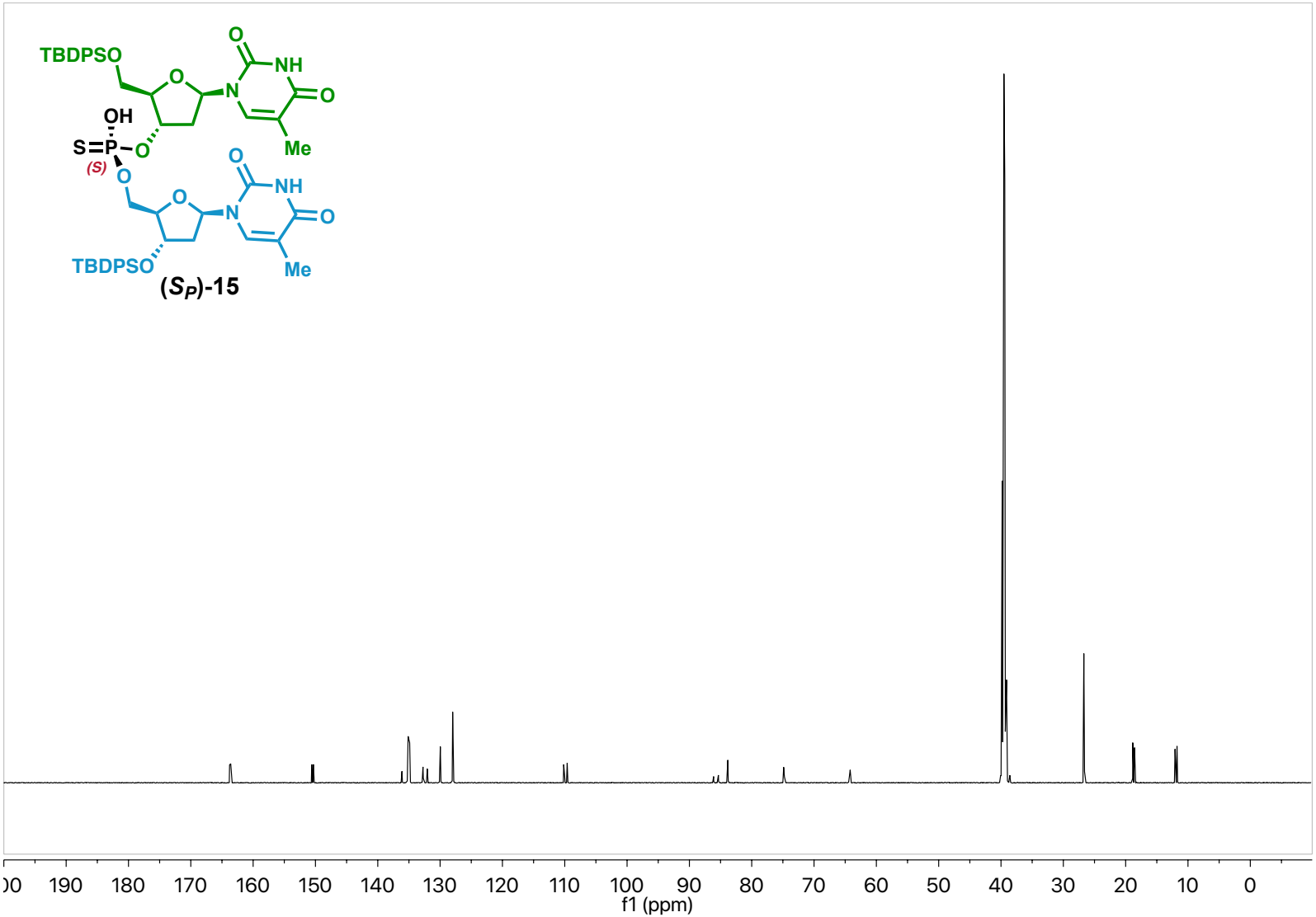
Compound (R_P)-14 ^{31}P NMR



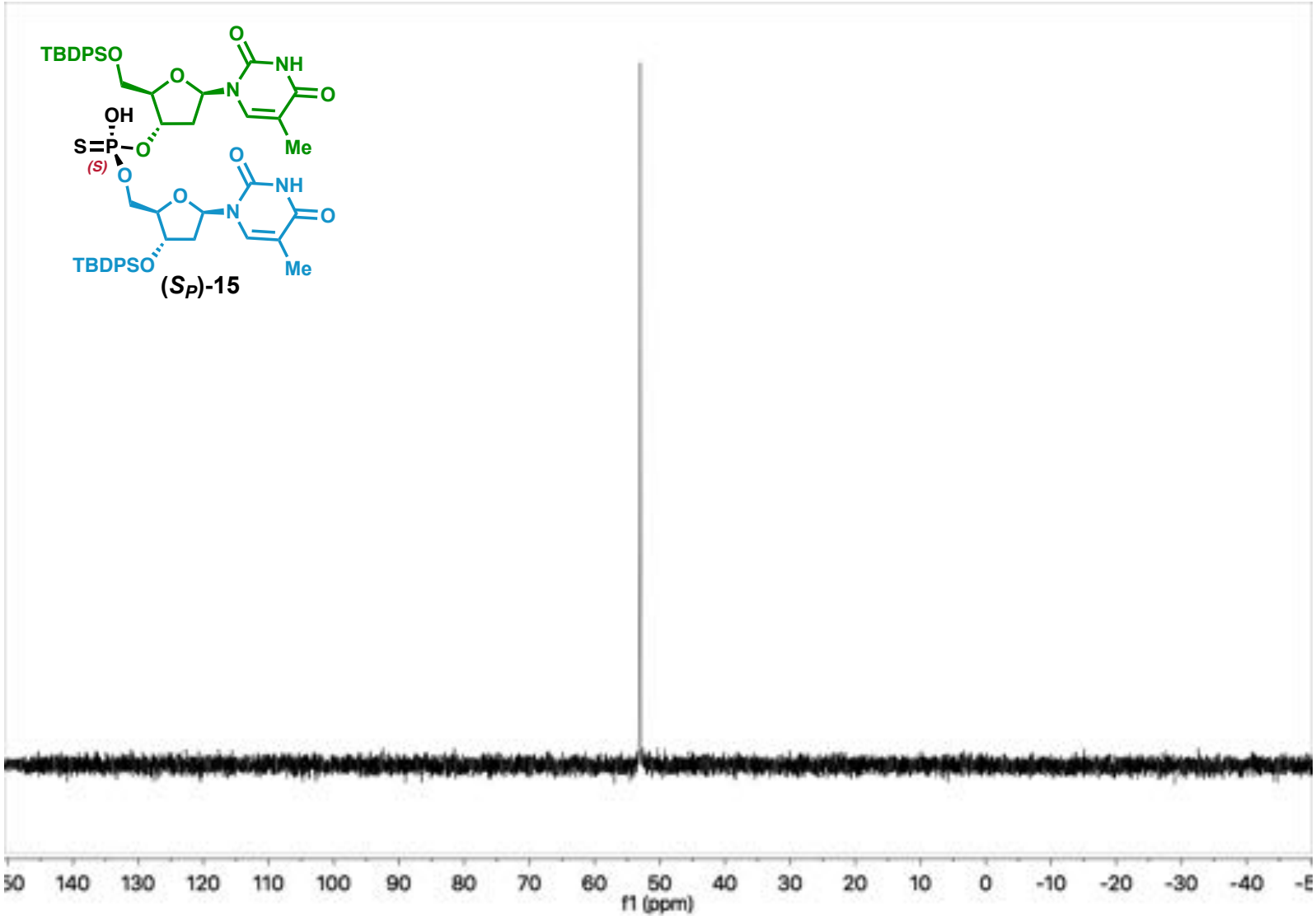
Compound (S_P)-15 ^{1}H NMR



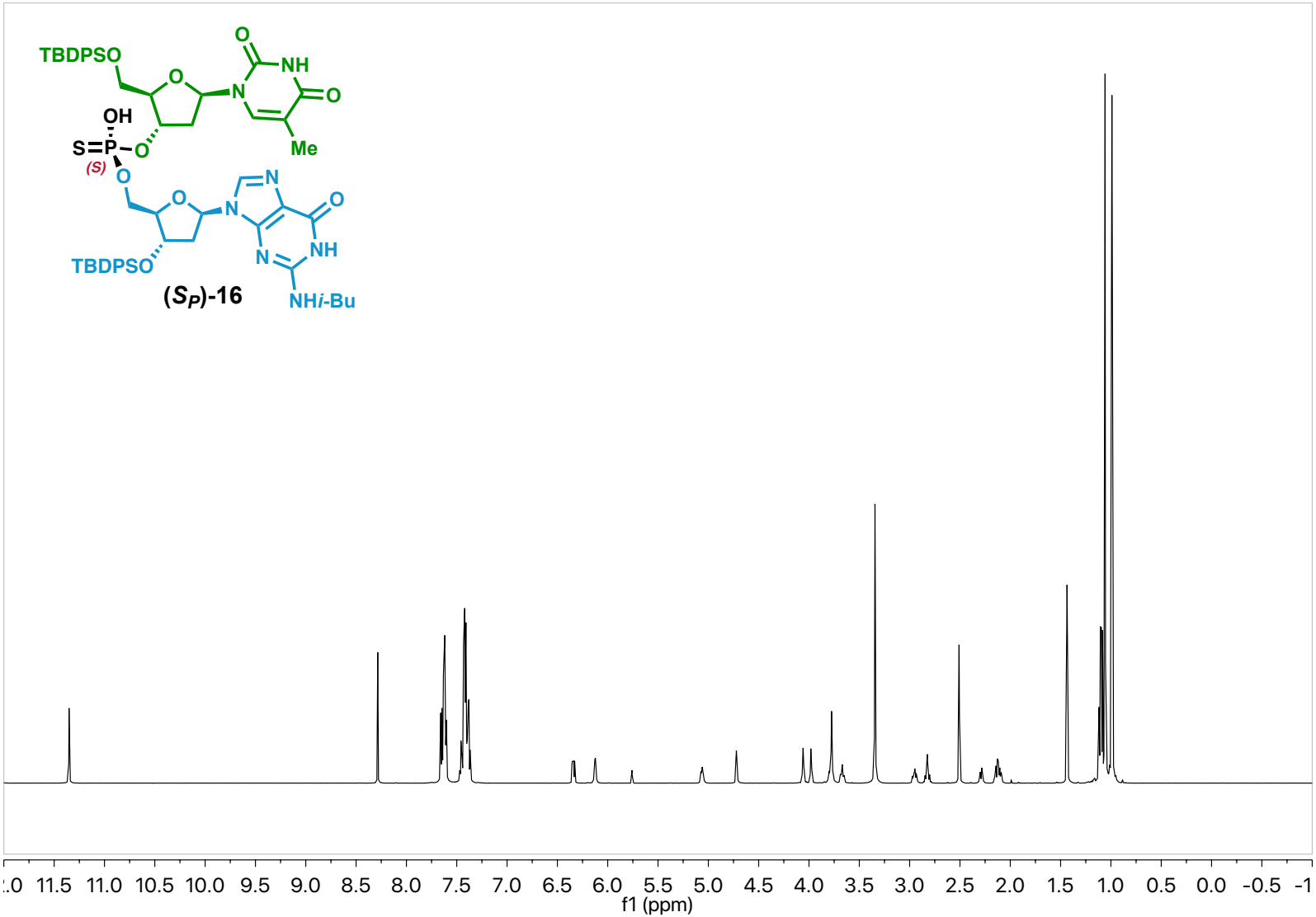
Compound (S_P)-15 ^{13}C NMR



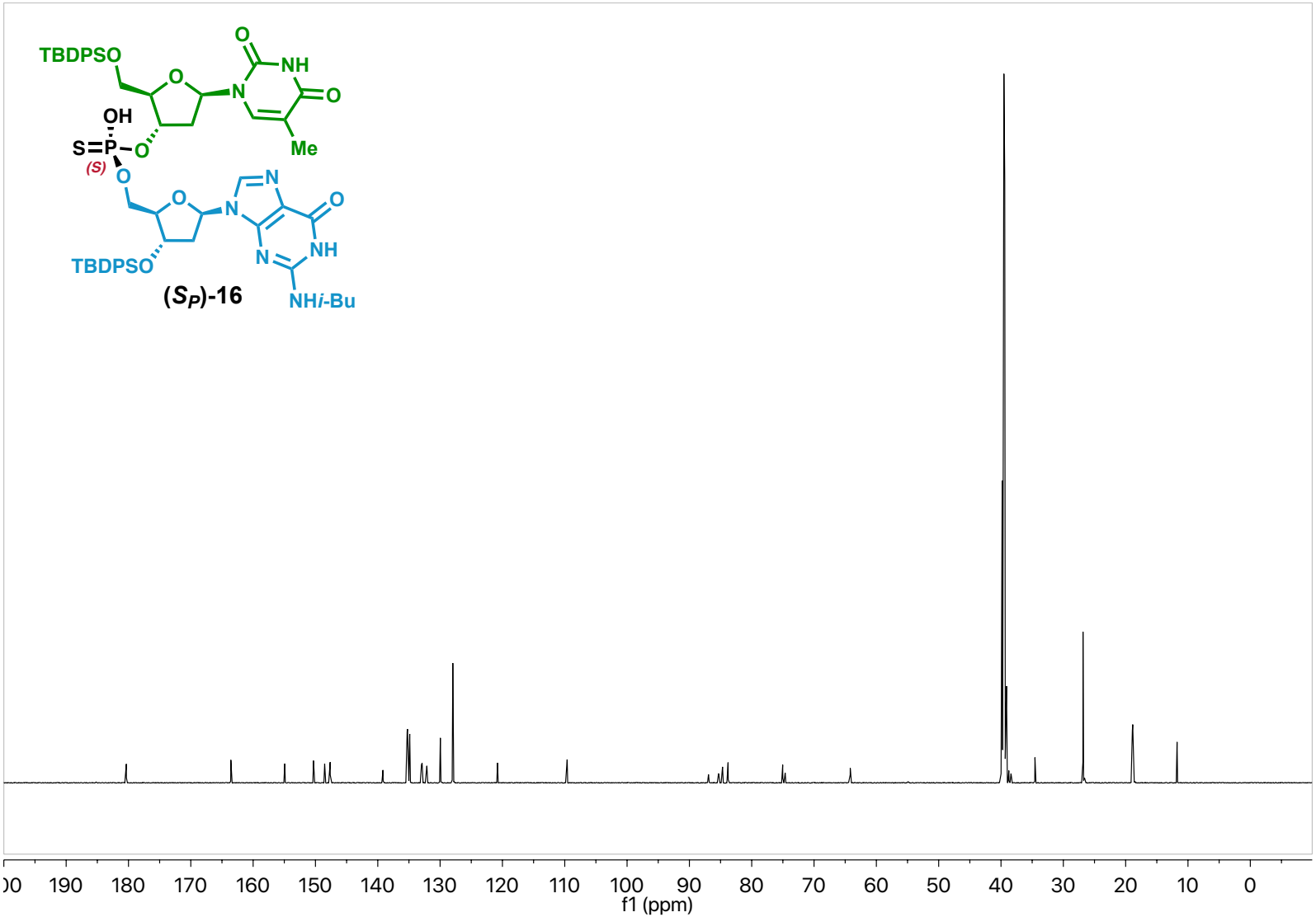
Compound (S_P)-15 ^{31}P NMR



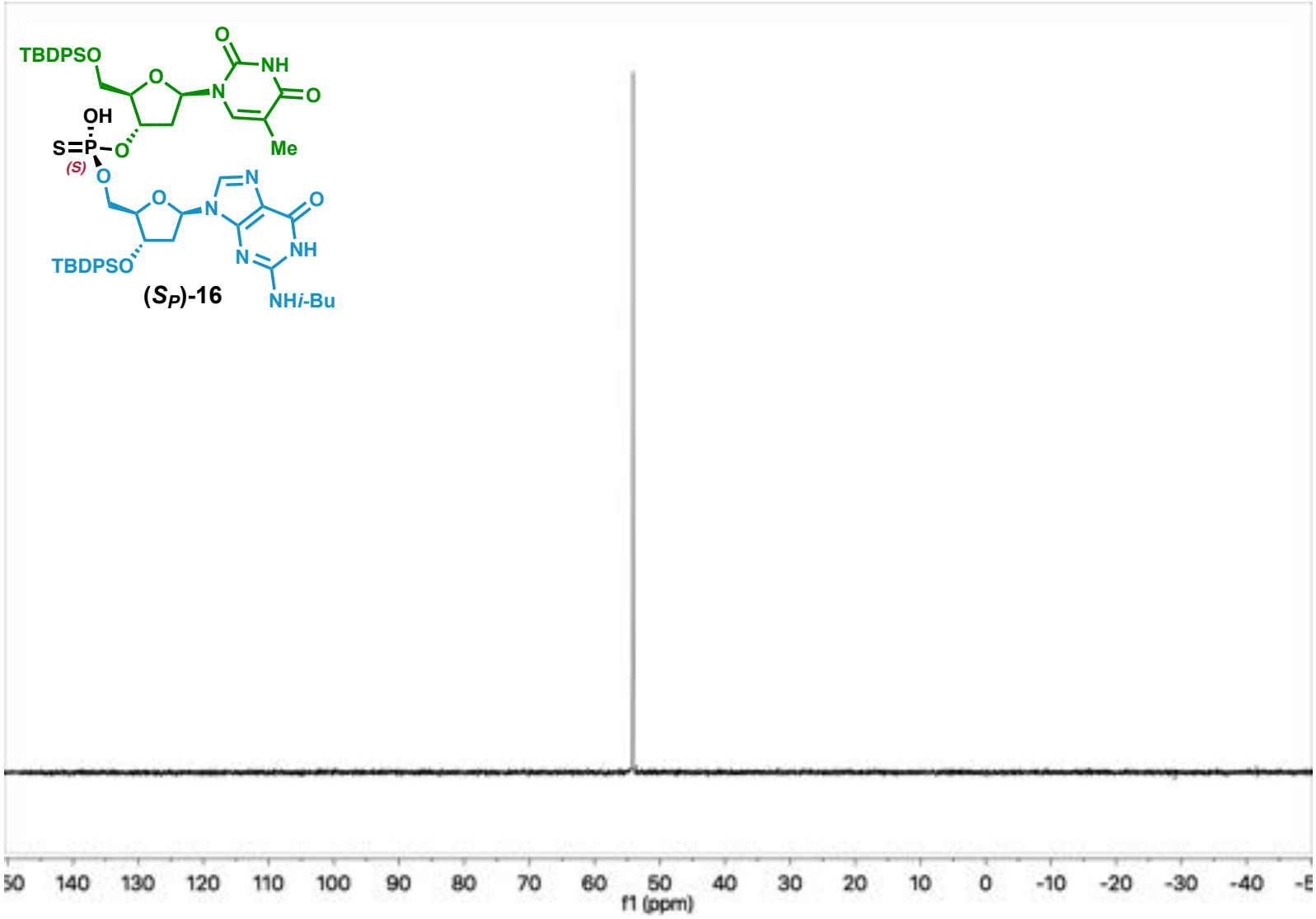
Compound (S_P)-16 ^{1}H NMR



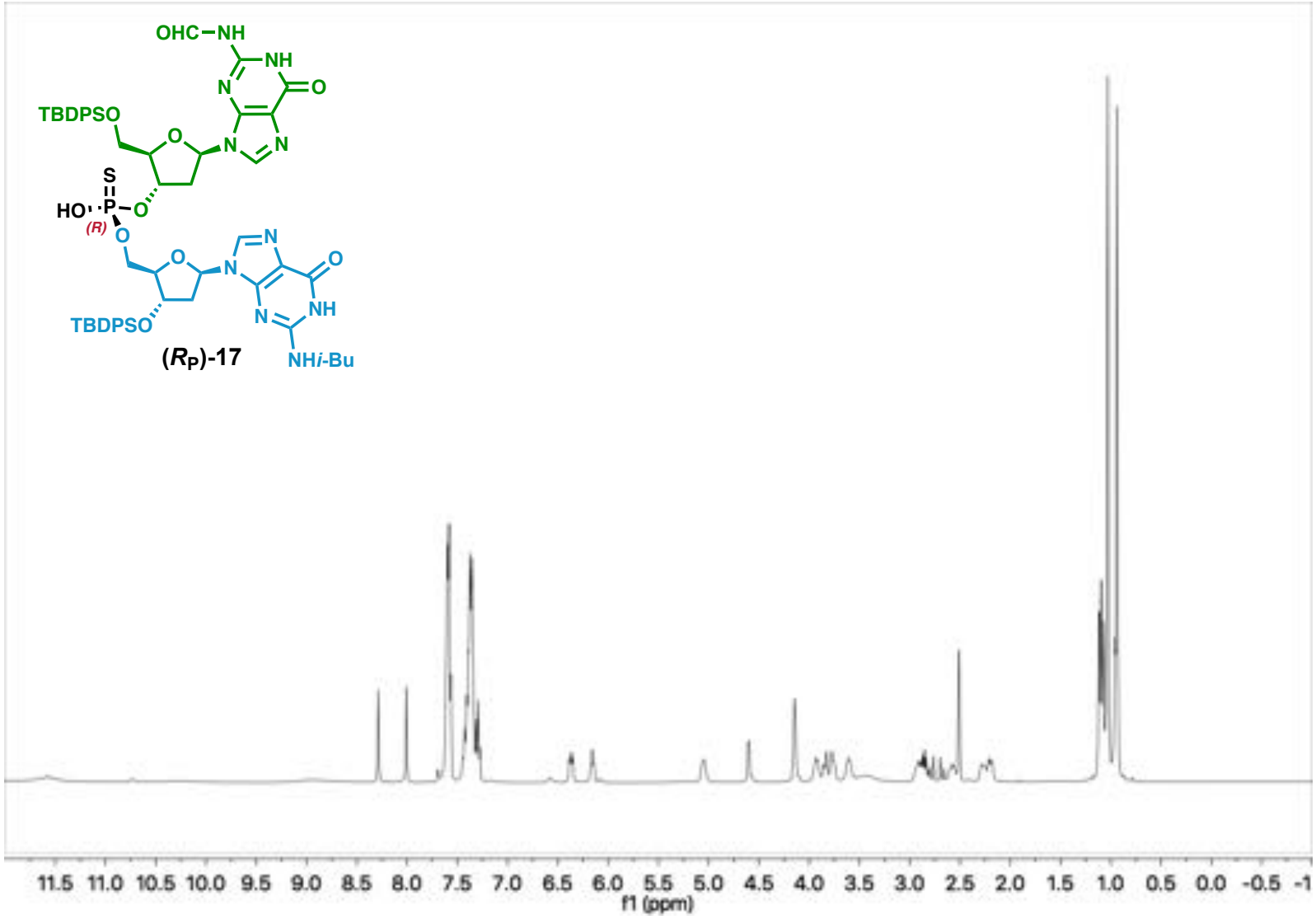
Compound (S_P)-16 ^{13}C NMR



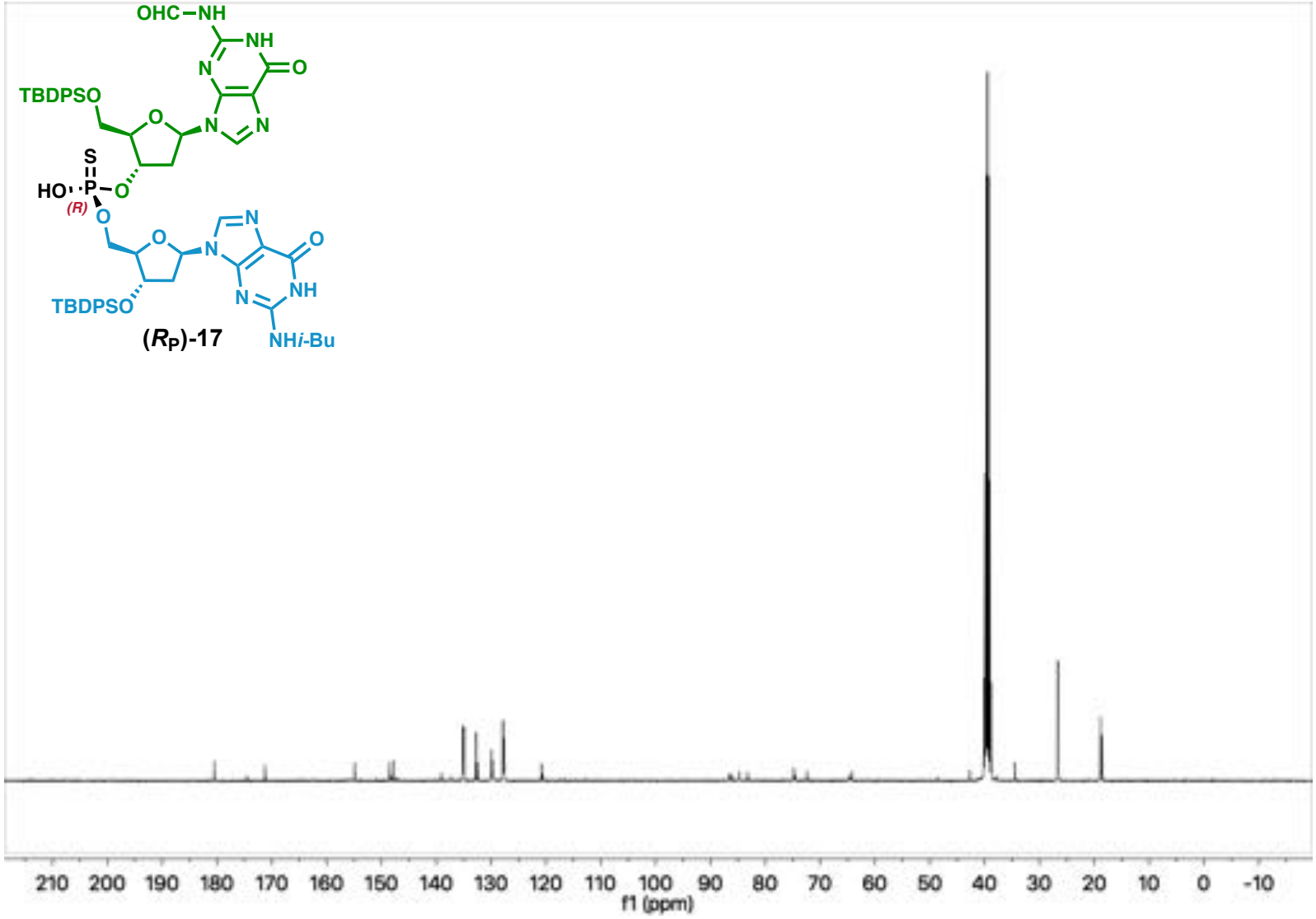
Compound (S_P)-16 ^{31}P NMR



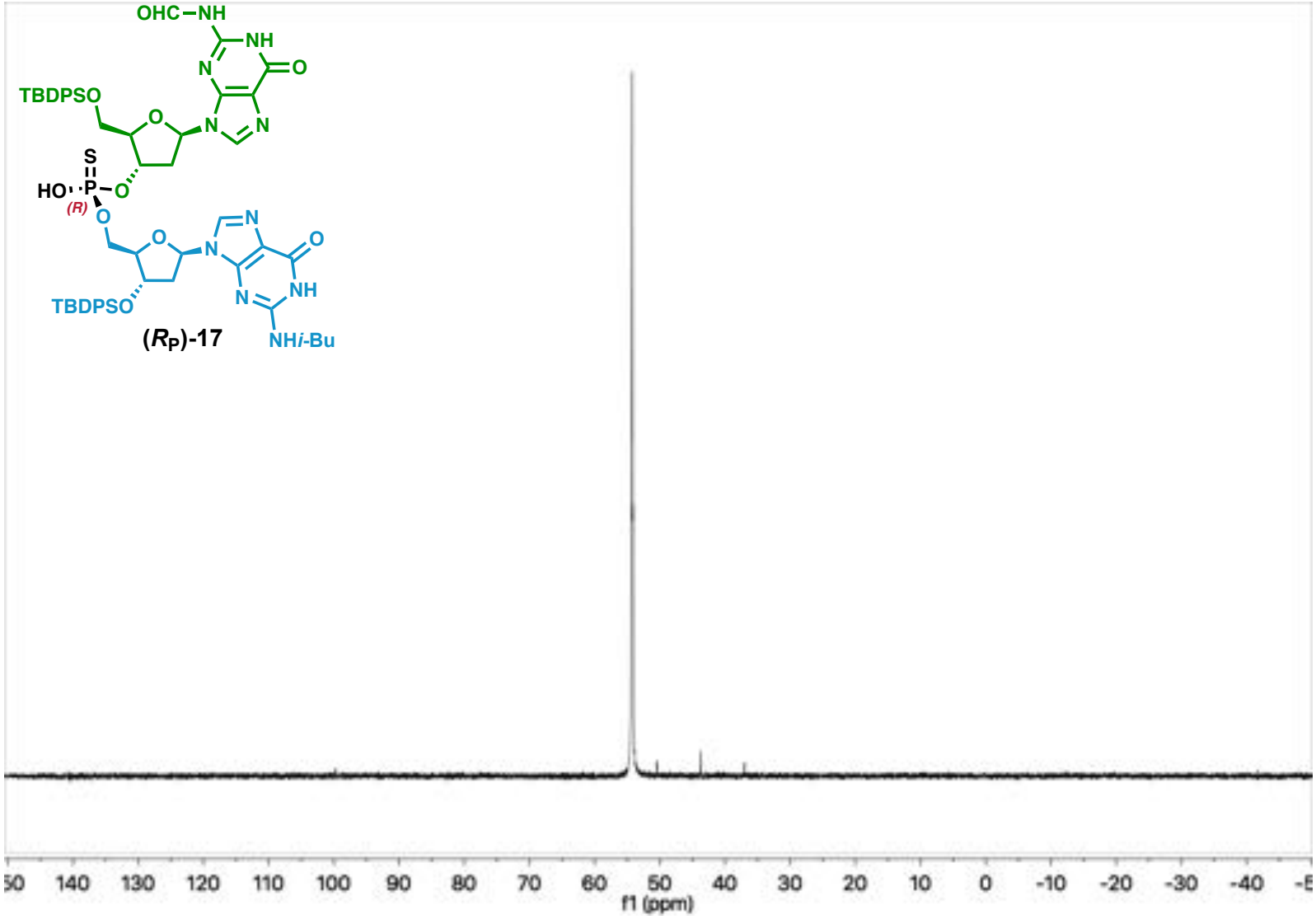
Compound (R_P)-17 ^{1}H NMR



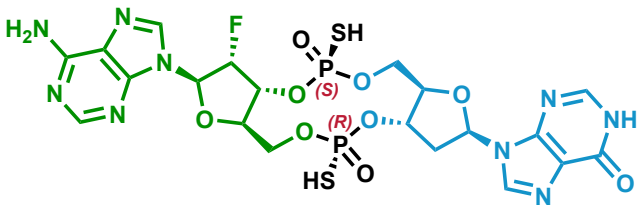
Compound (R_P)-17 ^{13}C NMR



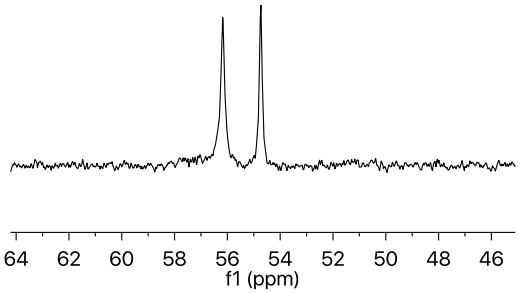
Compound (R_P)-17 ^{31}P NMR

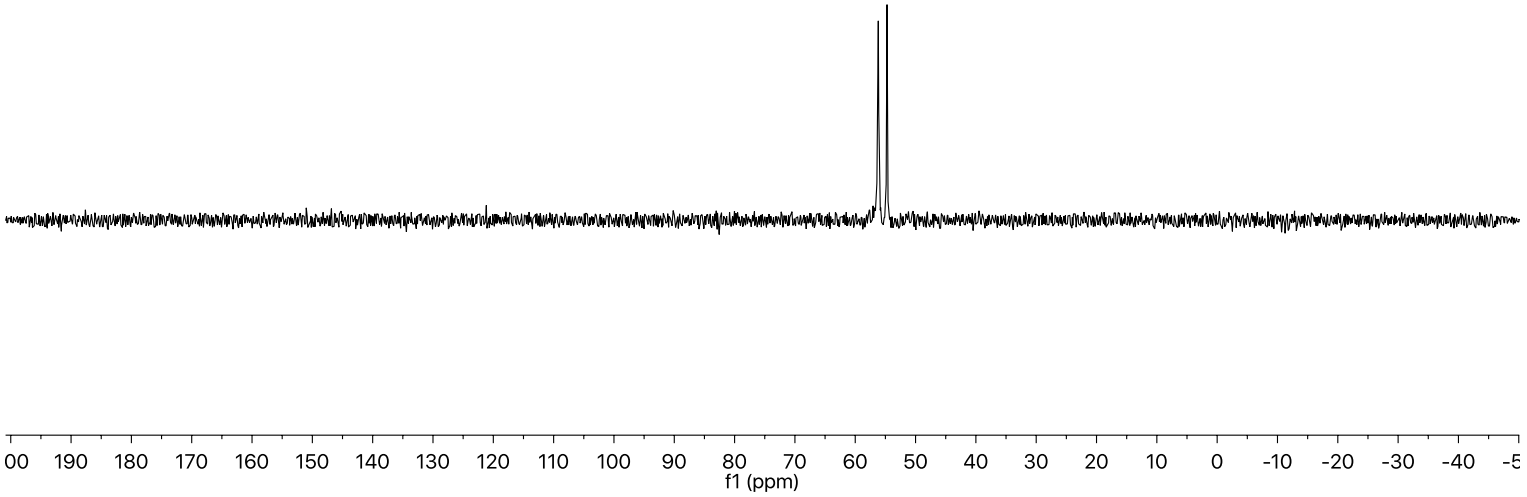


Compound (S_P, R_P)-18 ^{31}P NMR

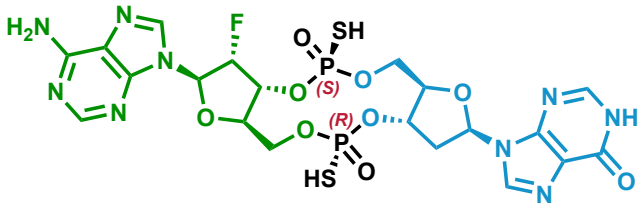


(S_P, R_P)-18

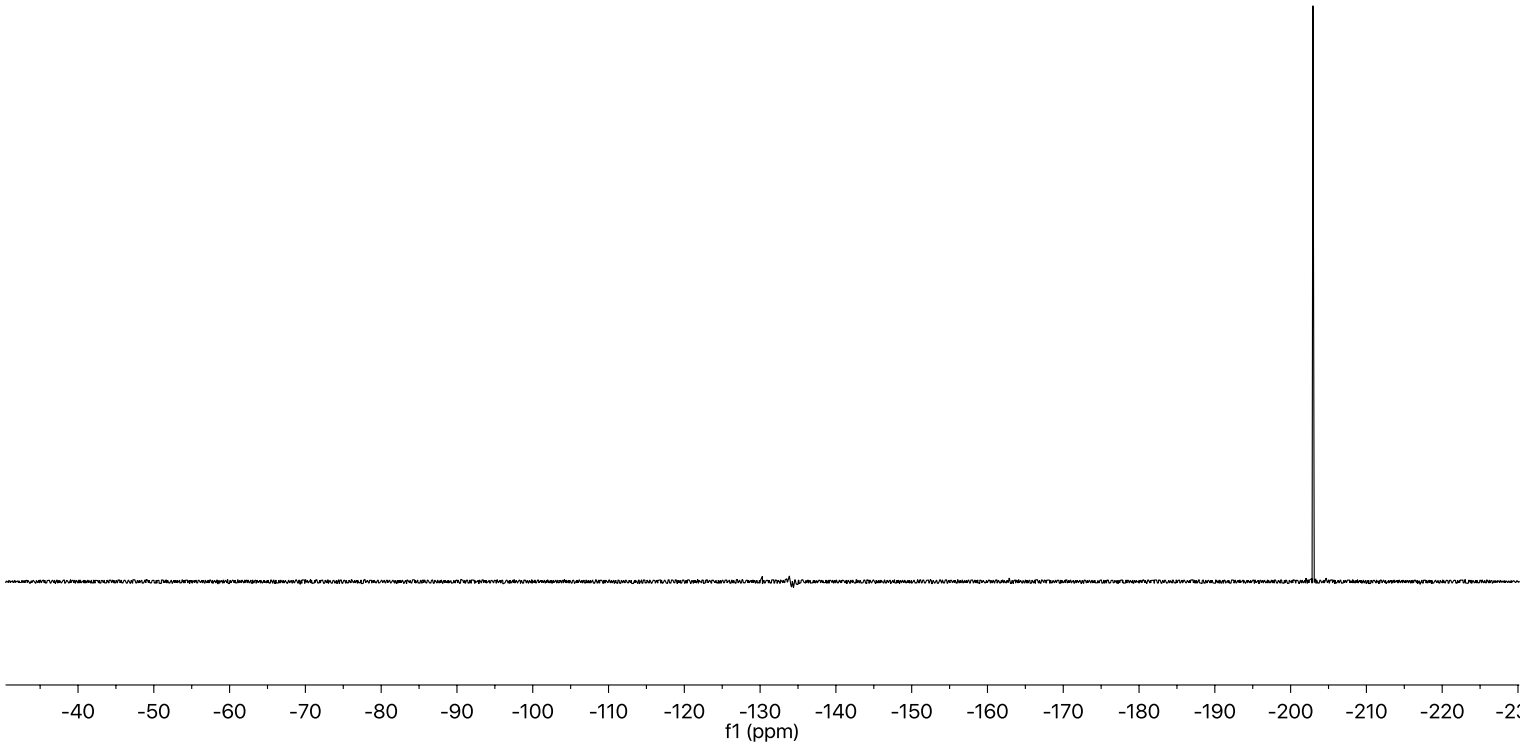




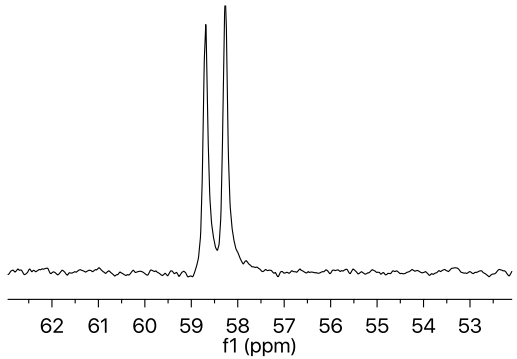
Compound (S_P, R_P)-18 ^{19}F NMR

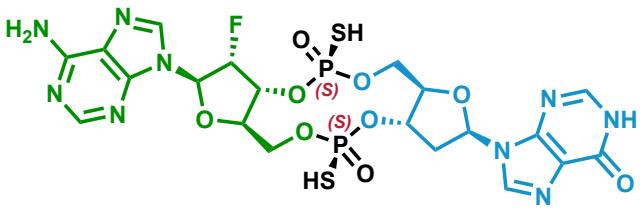


(S_P, R_P)-18

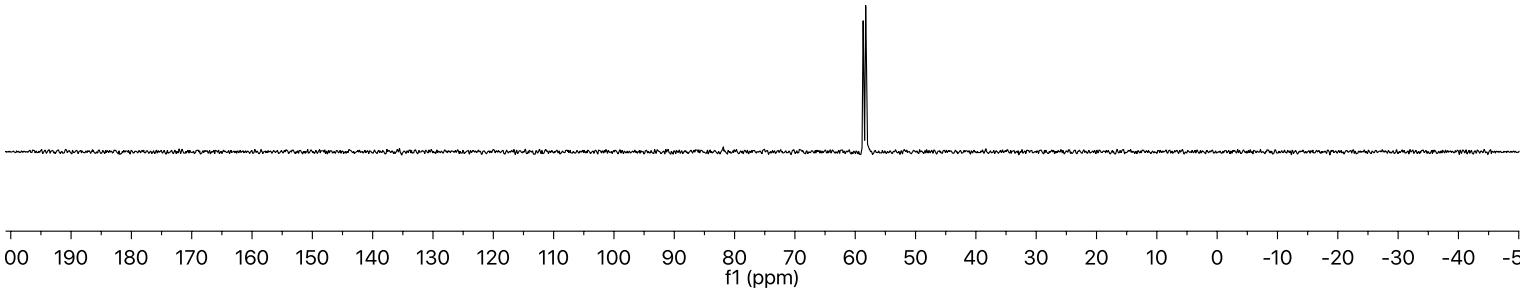


Compound (S_P, S_P)-18 ^{31}P NMR

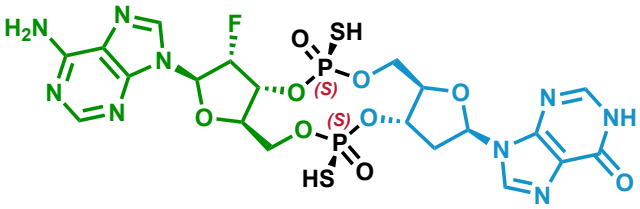




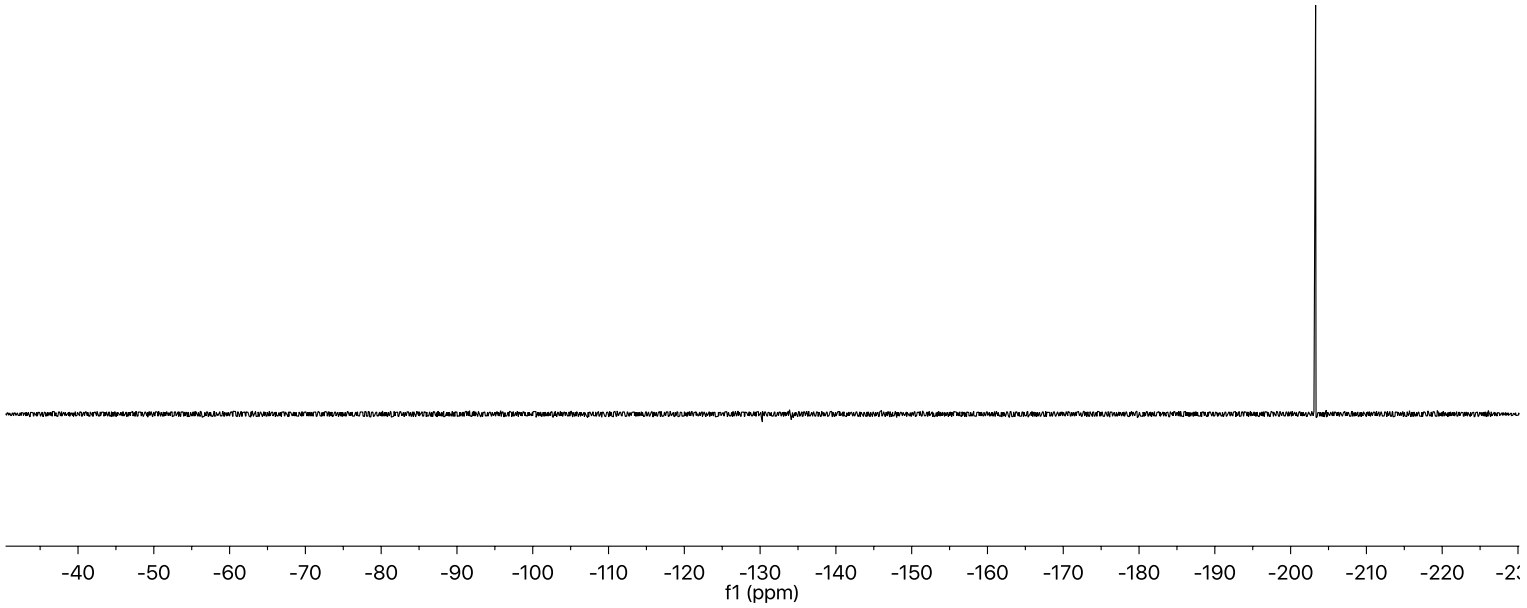
(S_P, S_P)-18



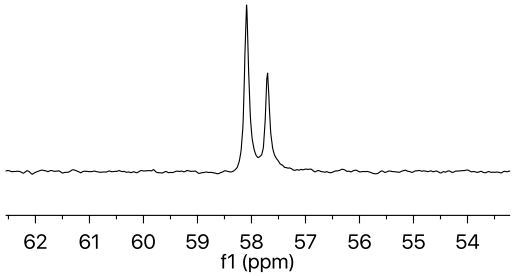
Compound (S_P, S_P)-18 ^{19}F NMR

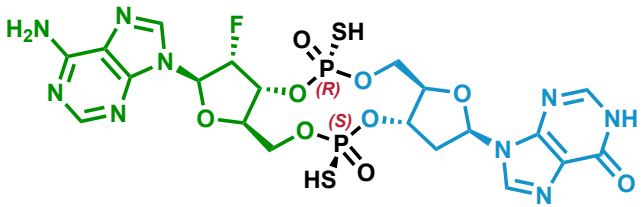


(S_P, S_P)-18

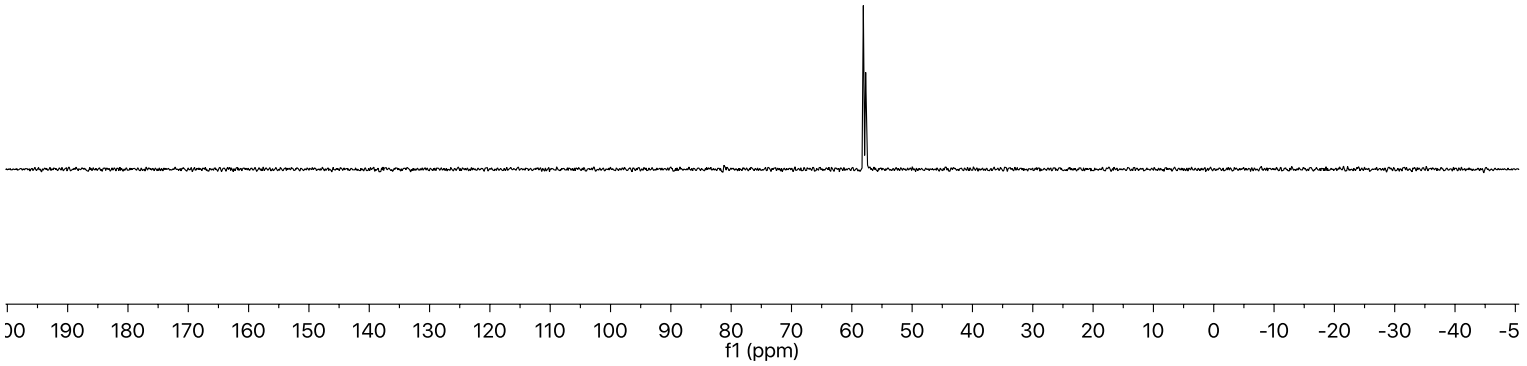


Compound (R_P, S_P)-18 ^{31}P NMR

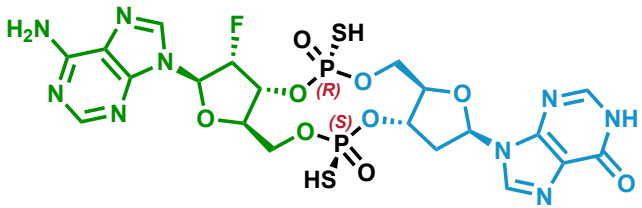




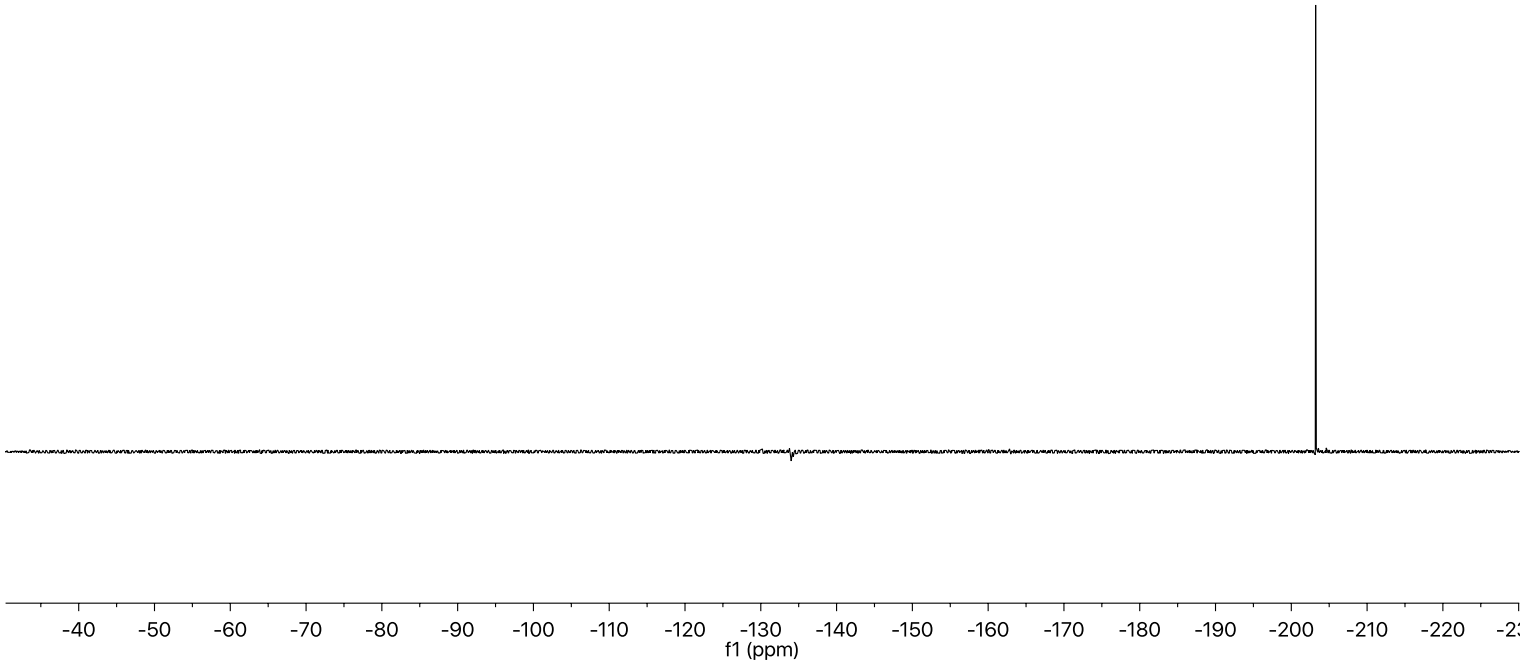
(R_P, S_P)-18



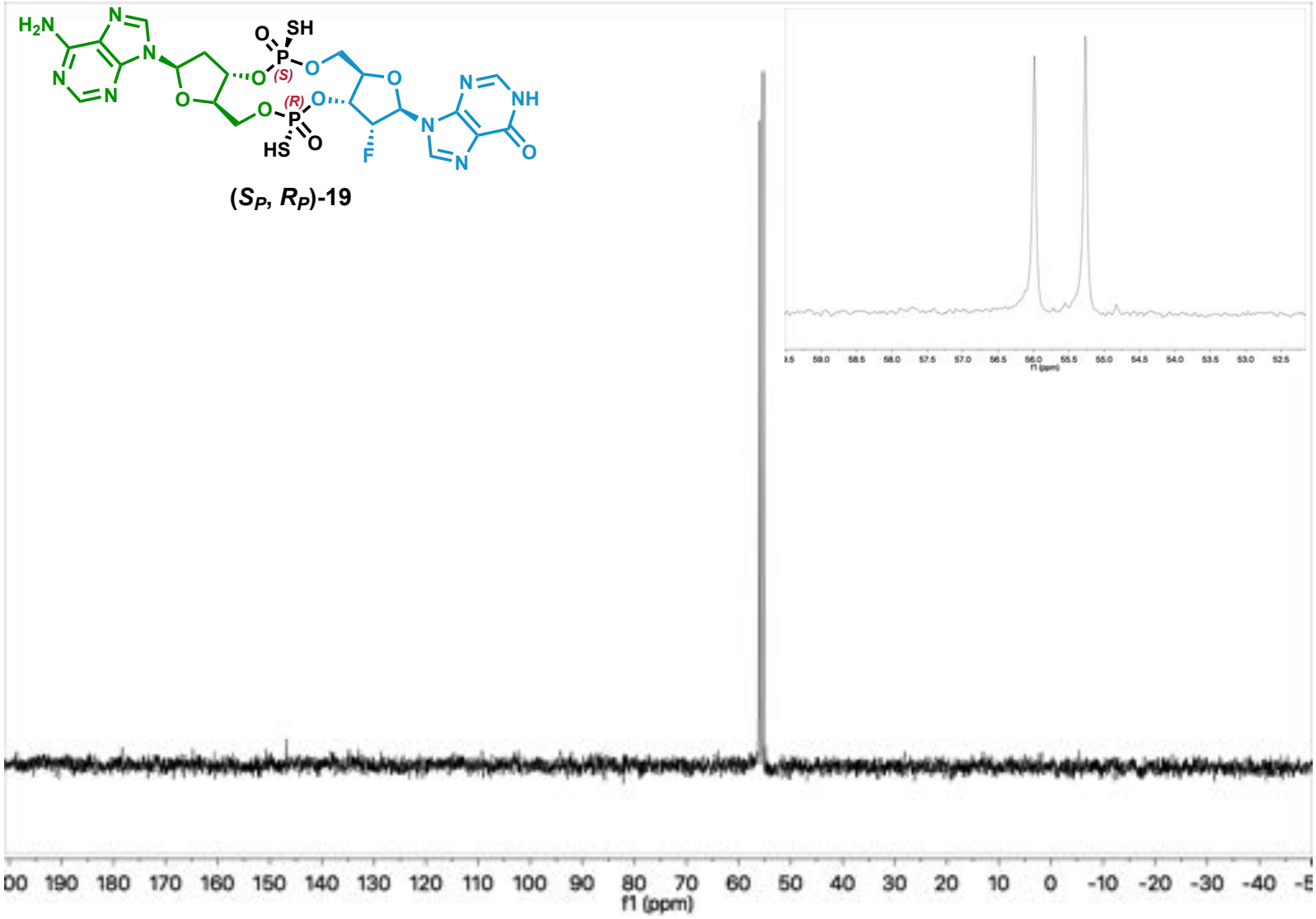
Compound (R_P, S_P)-18 ^{19}F NMR



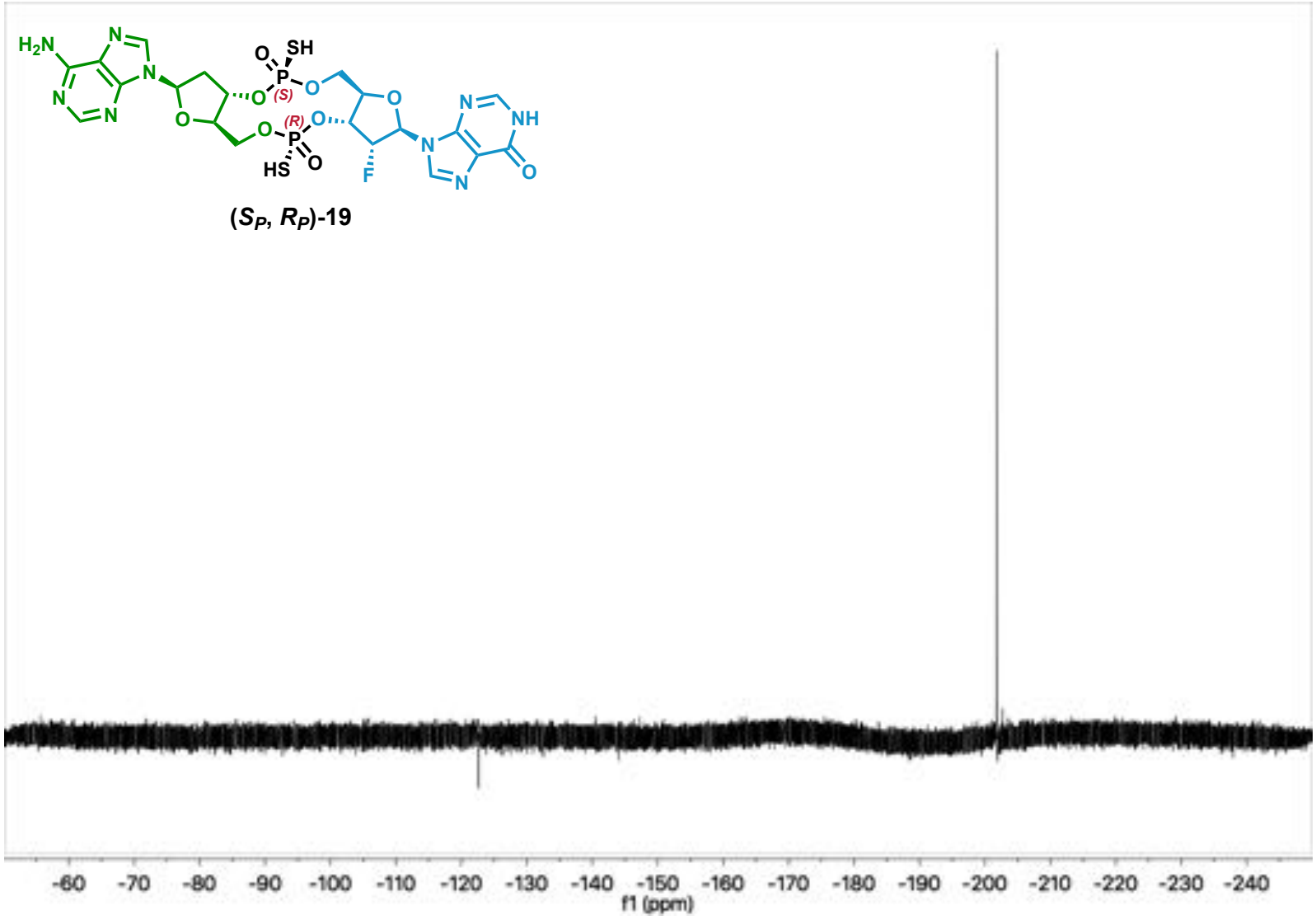
(R_P, S_P)-18



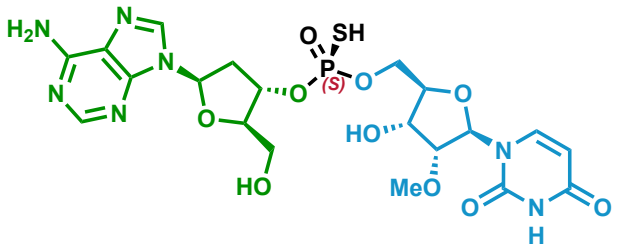
Compound (R_P, S_P)-19 ^{31}P NMR



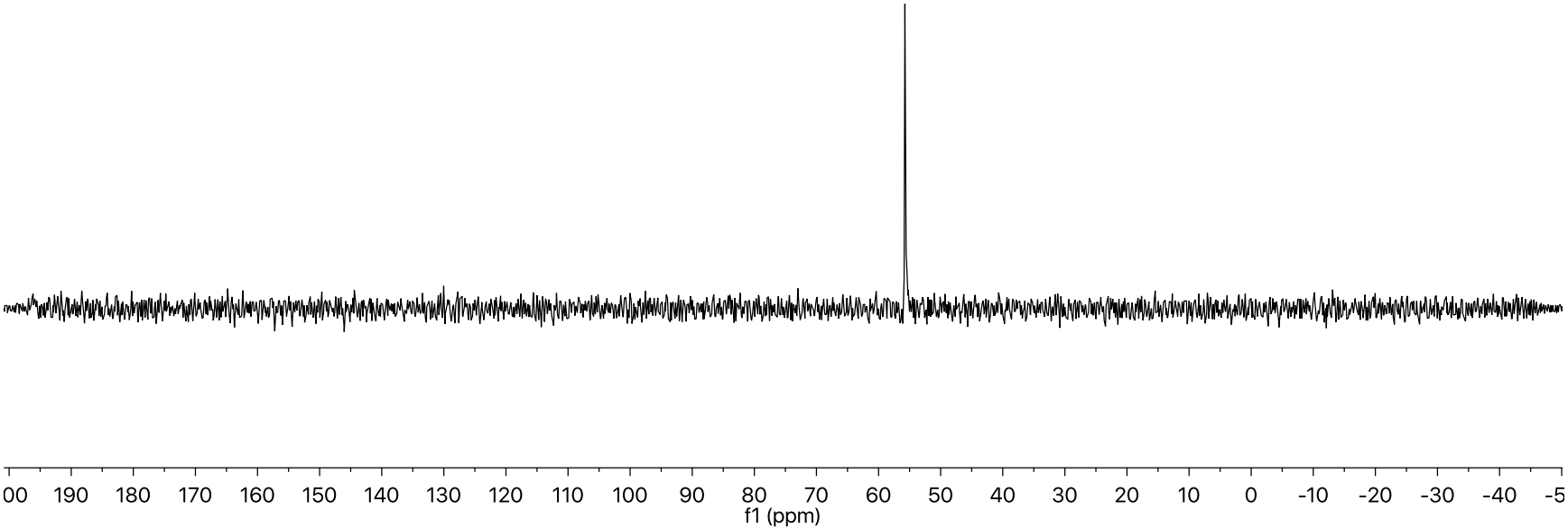
Compound (S_P, R_P)-19 ^{19}F NMR



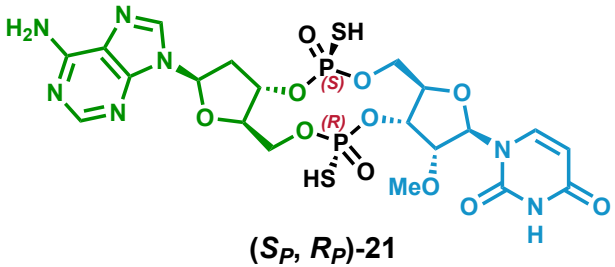
Compound (S_P)-20 ^{31}P NMR



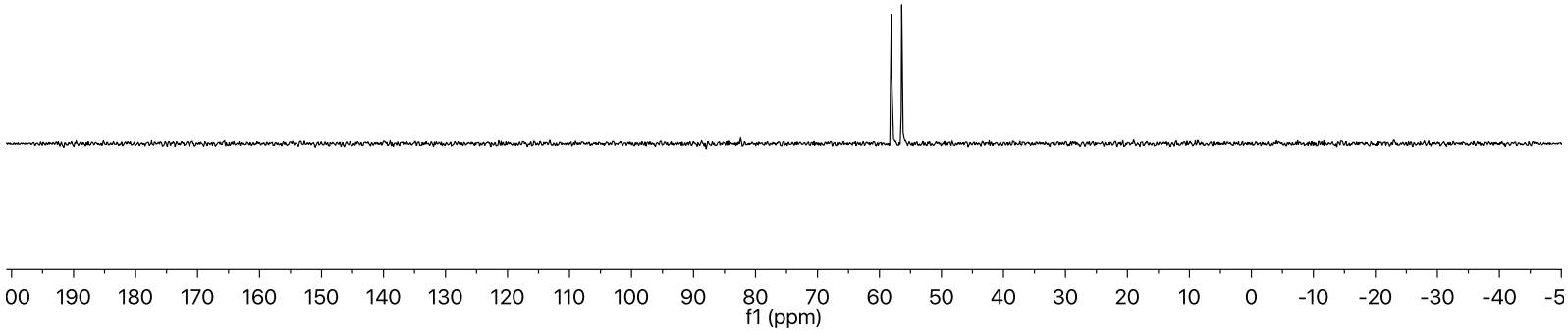
(S_P)-20



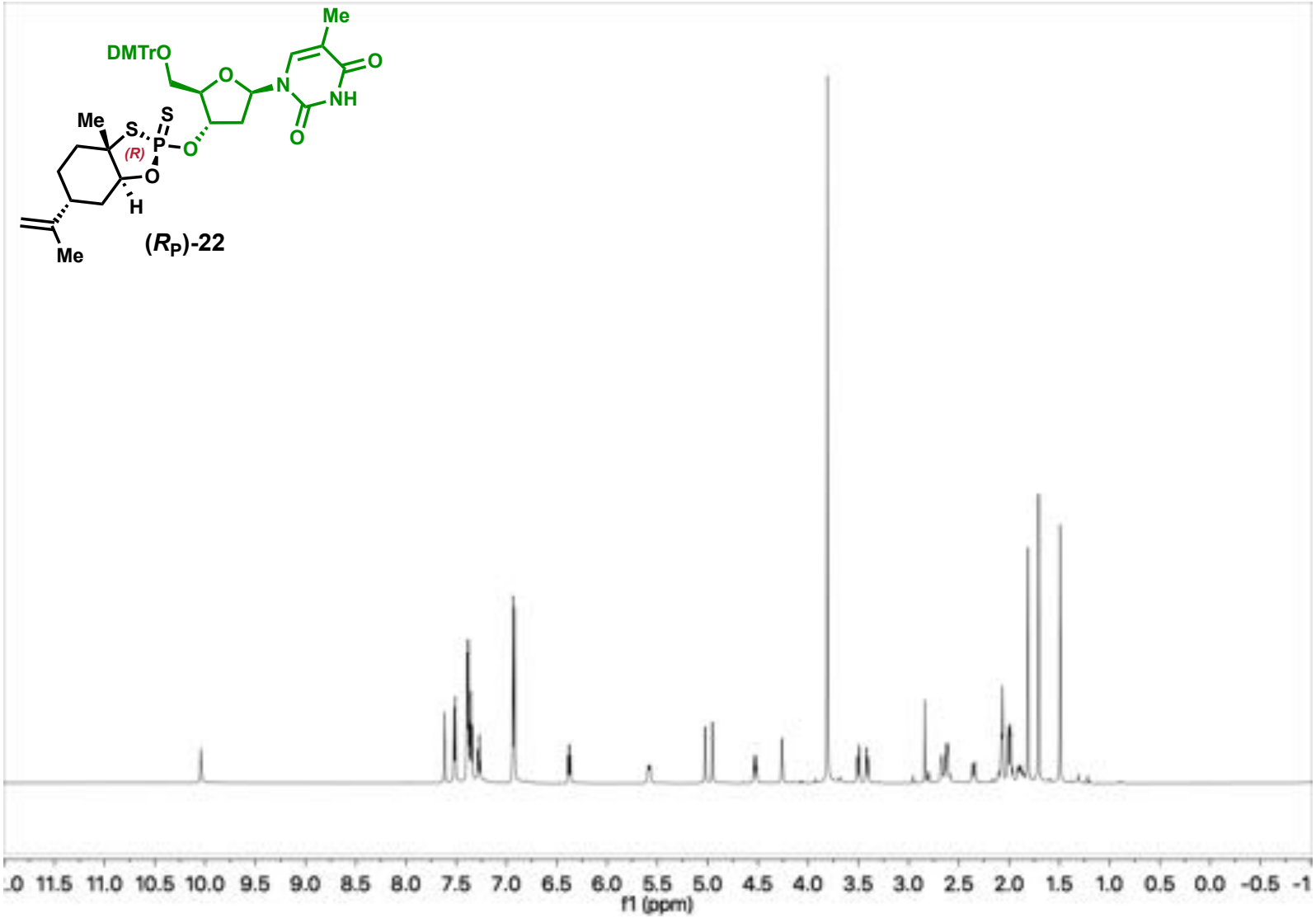
Compound (S_P, R_P)-21 ^{31}P NMR



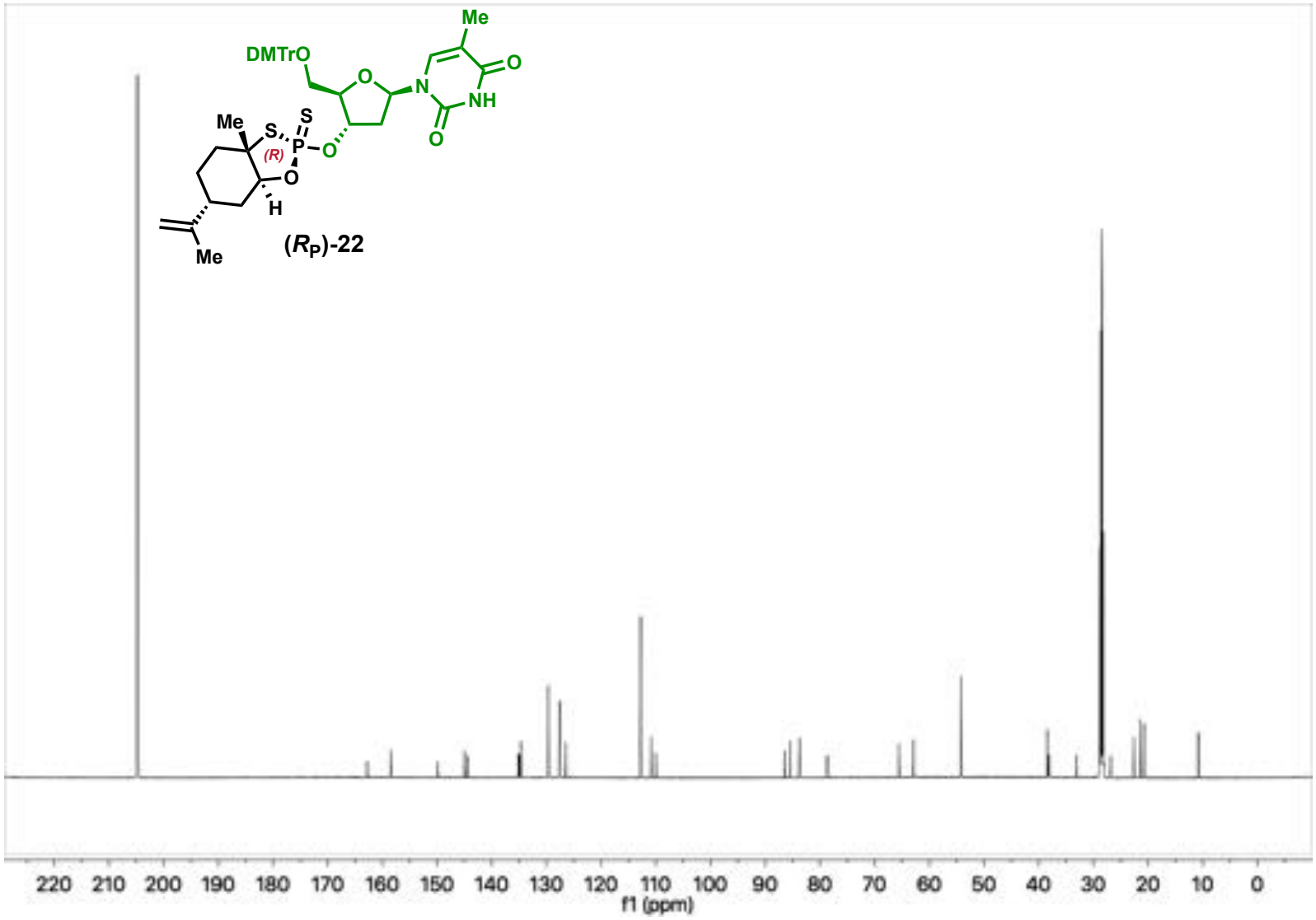
(S_P, R_P)-21



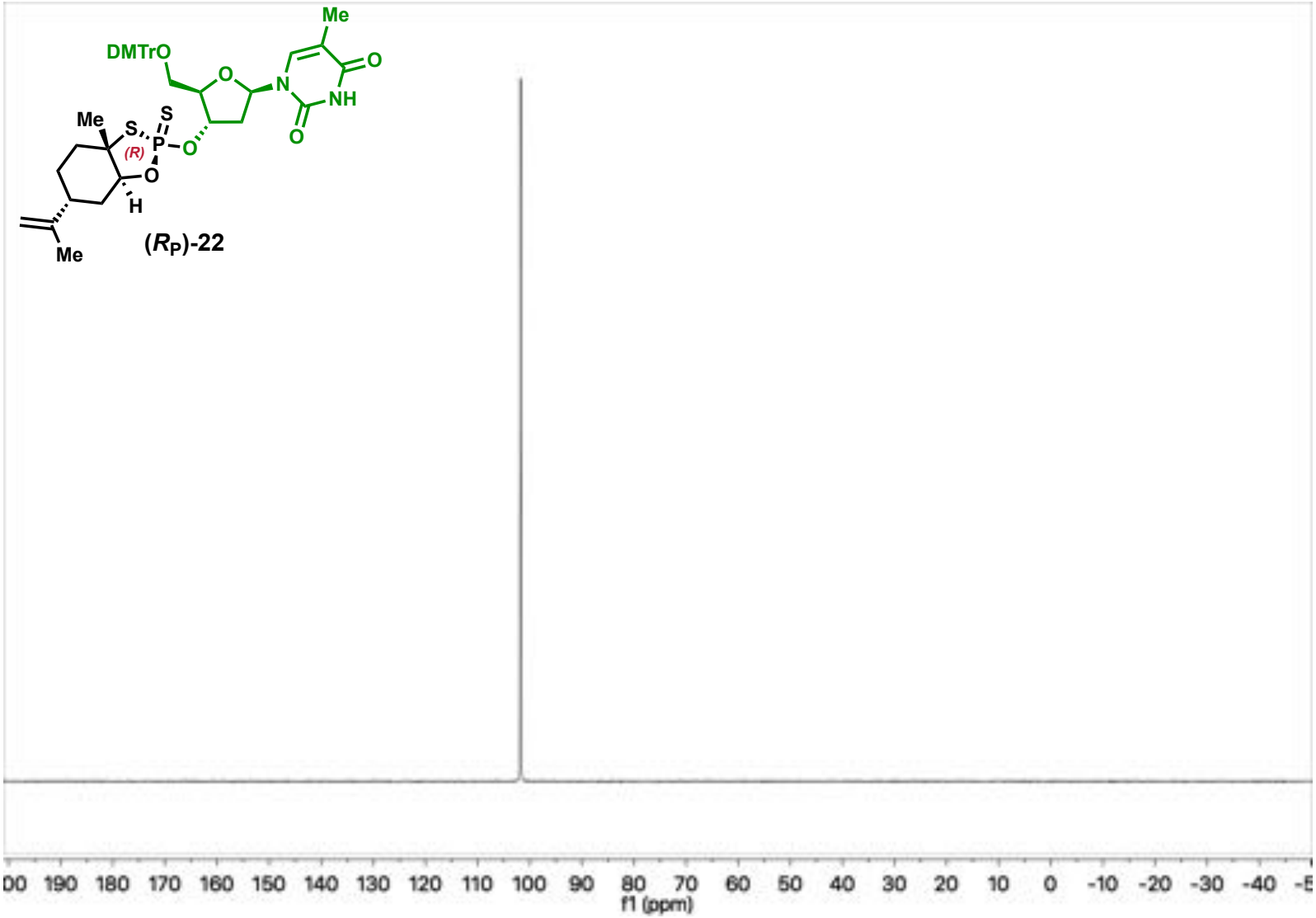
Compound (R_P)-22 ^{1}H NMR



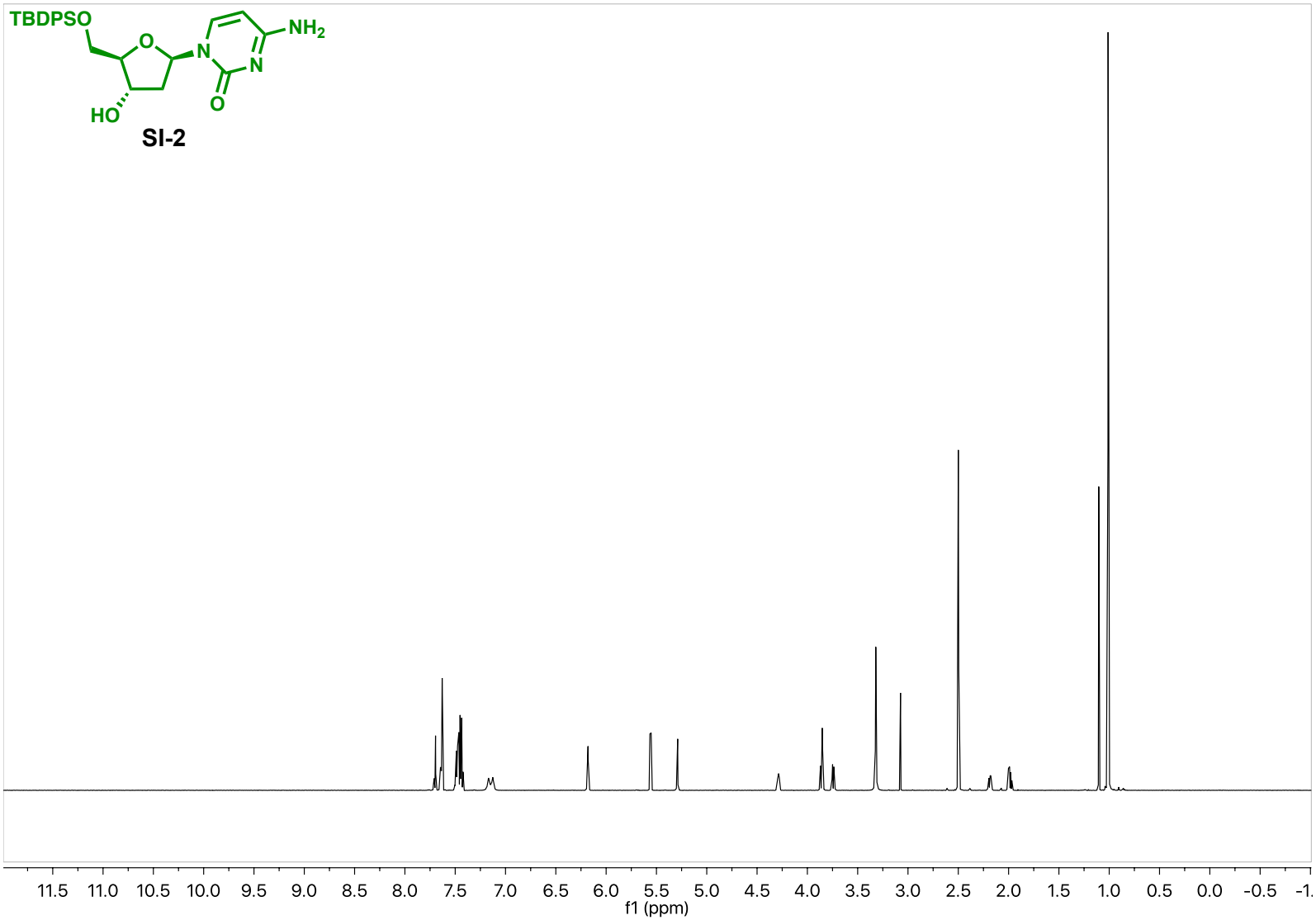
Compound (R_P)-22 ^{13}C NMR



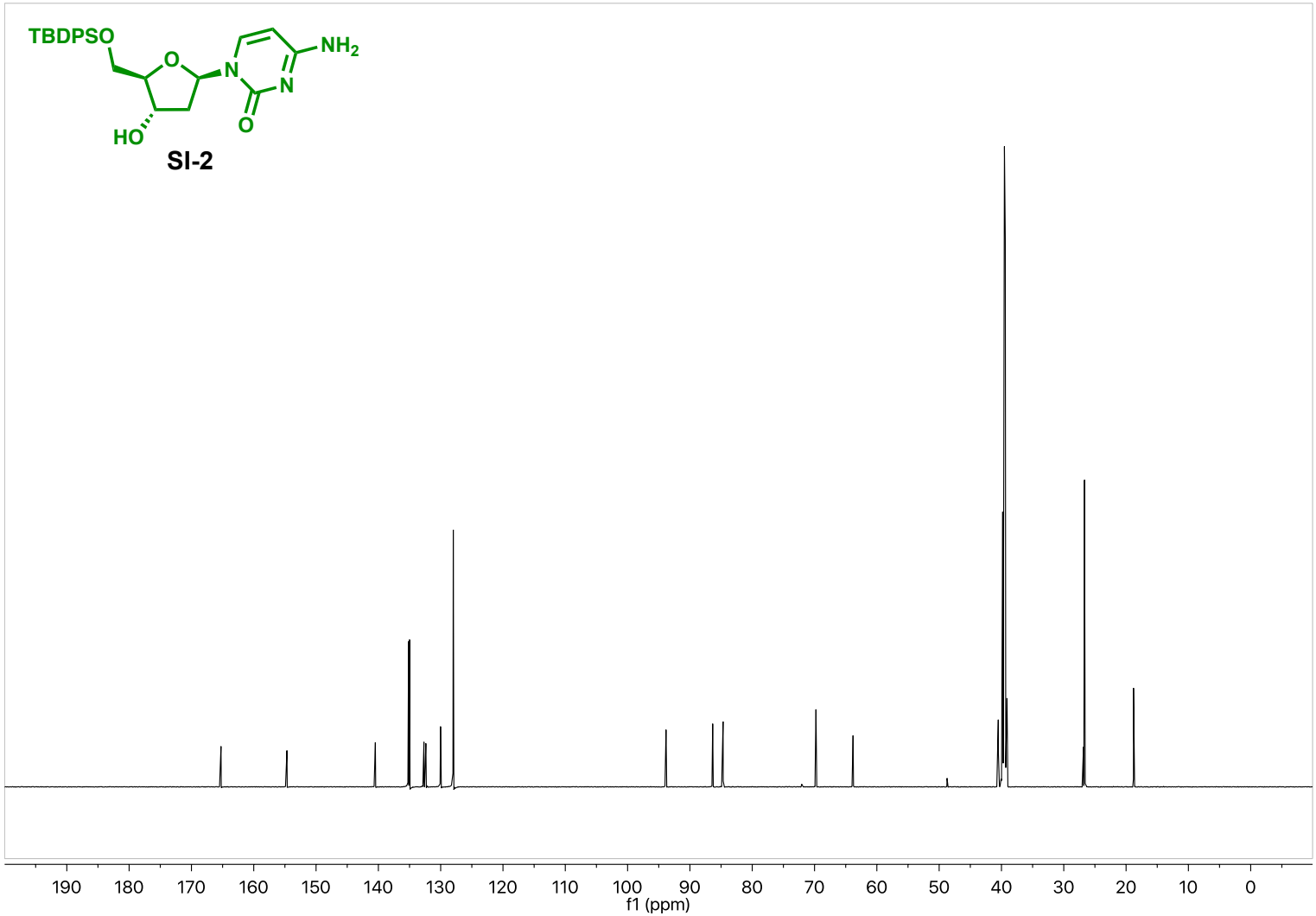
Compound (R_P)-22 ^{31}P NMR



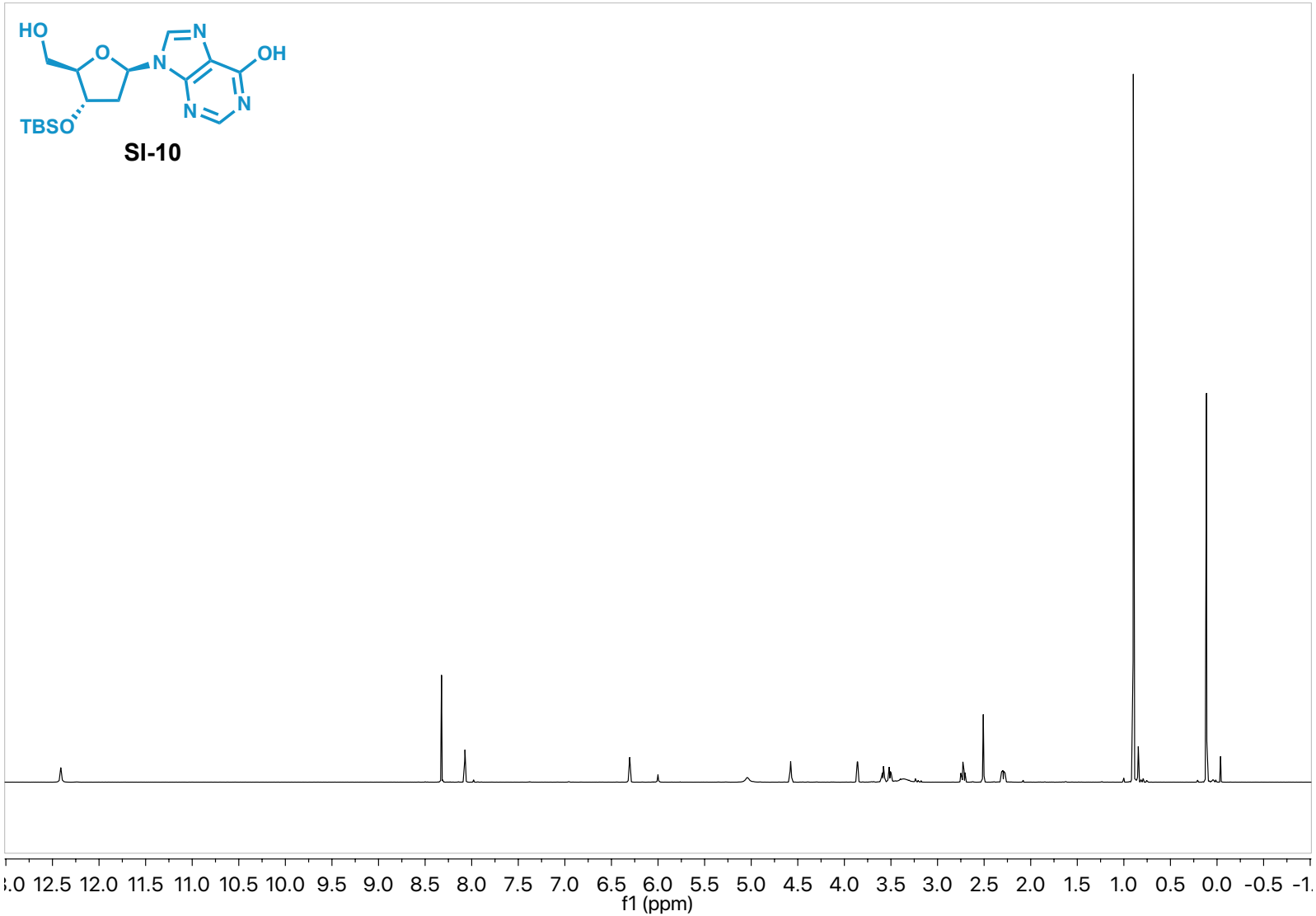
Compound SI-2 ^{1}H NMR



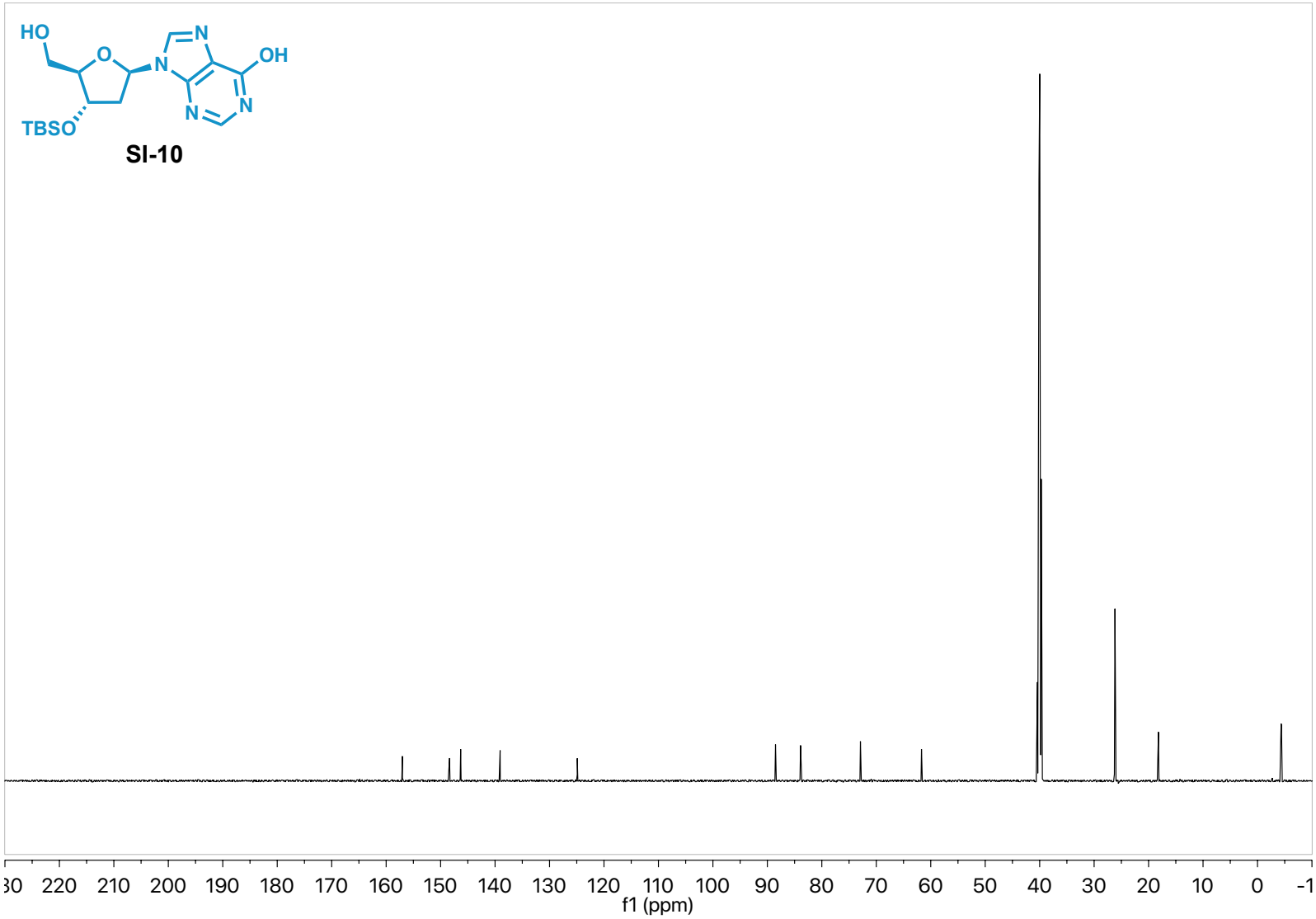
Compound SI-2 ^{13}C NMR



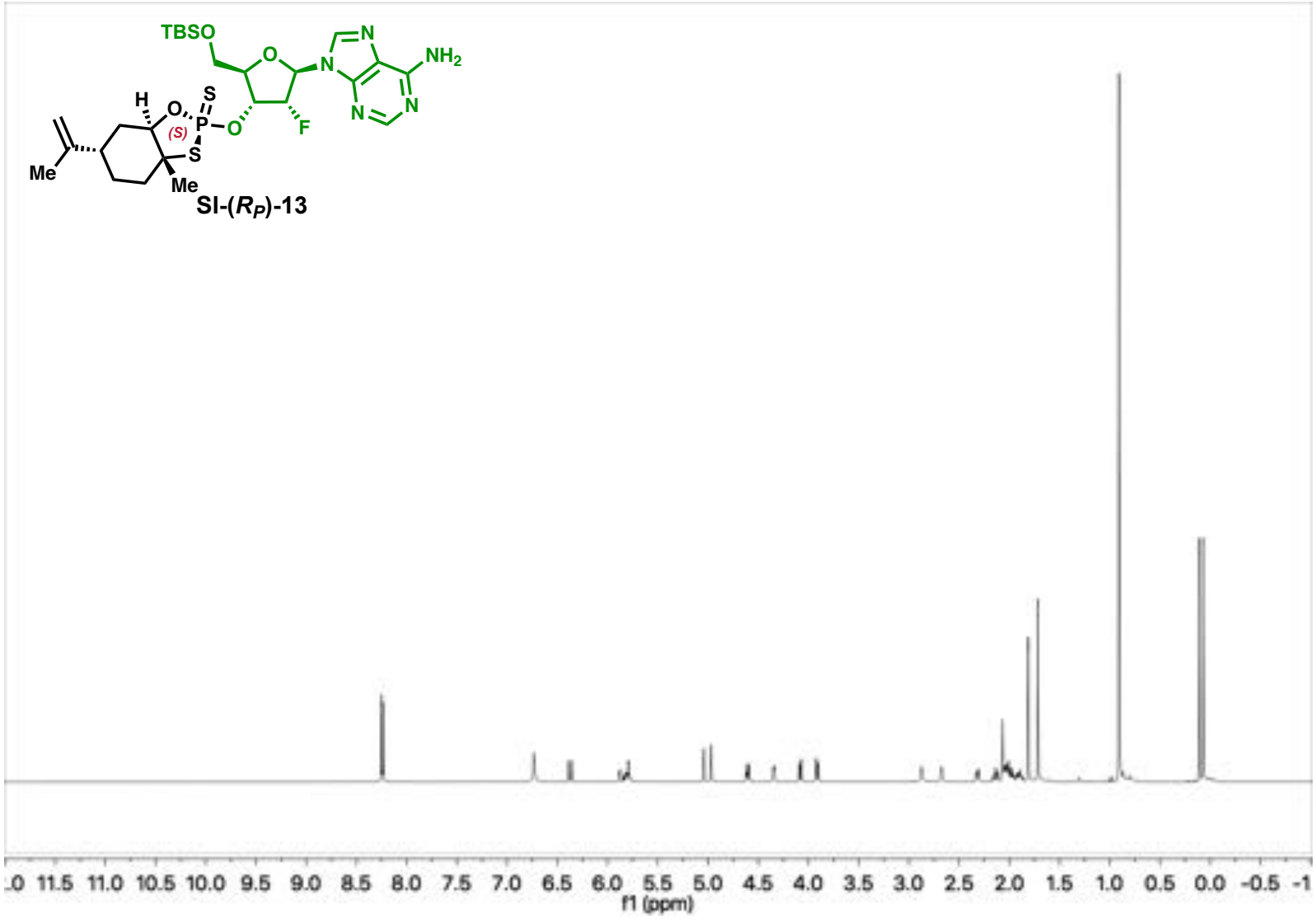
Compound SI-10 ^{1}H NMR



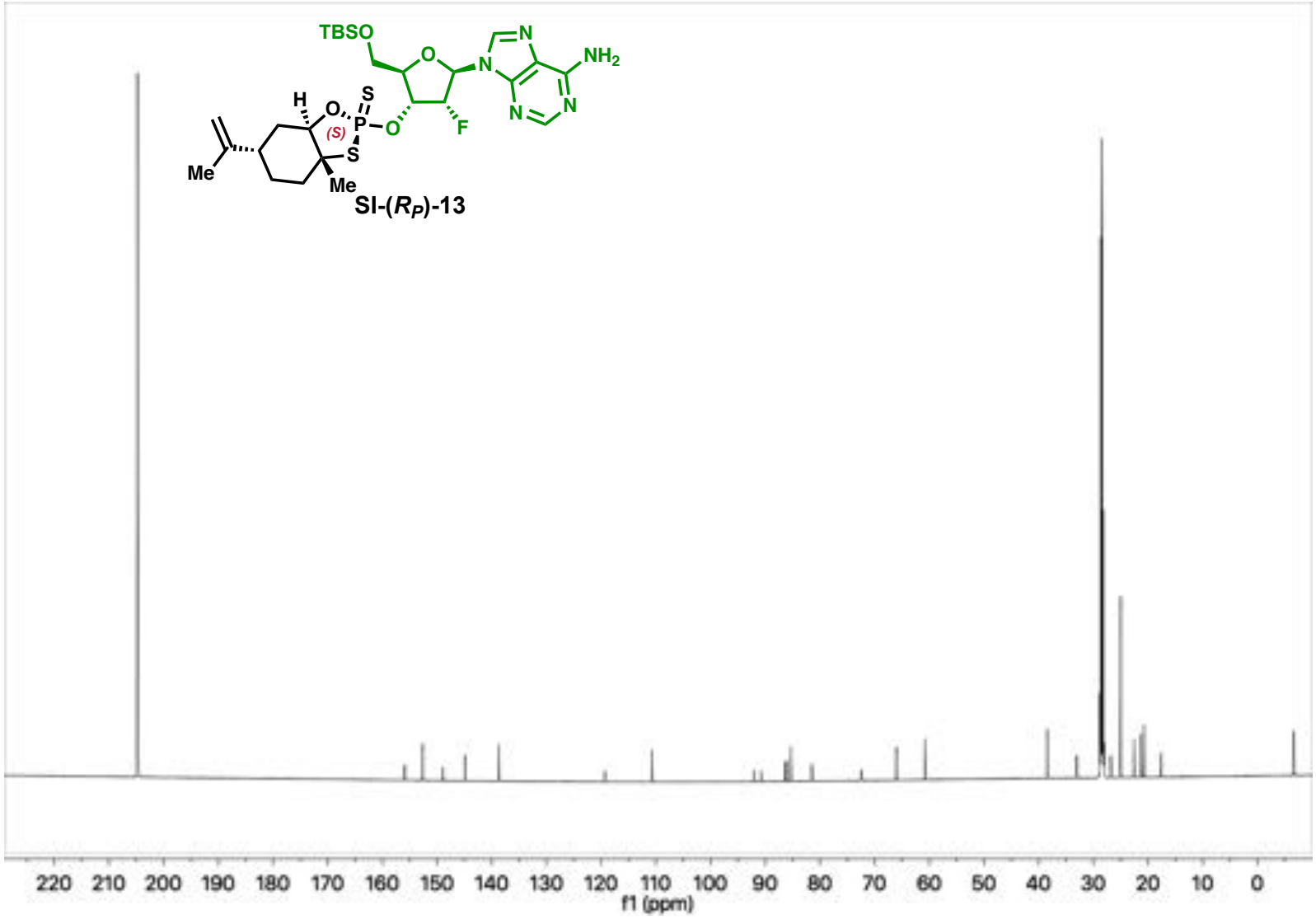
Compound SI-10 ^{13}C NMR



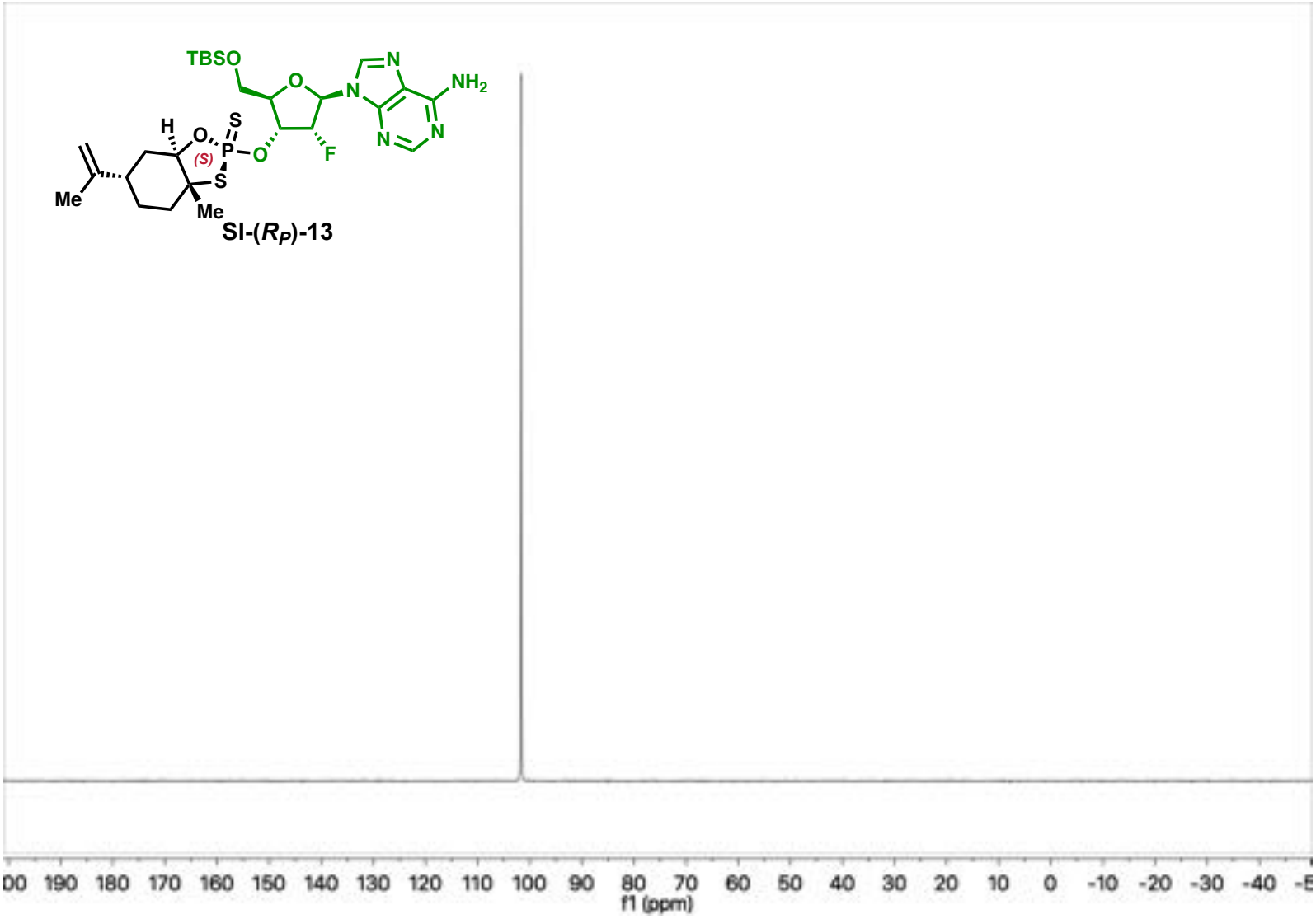
Compound SI-(R_P)-13 ^{1}H NMR



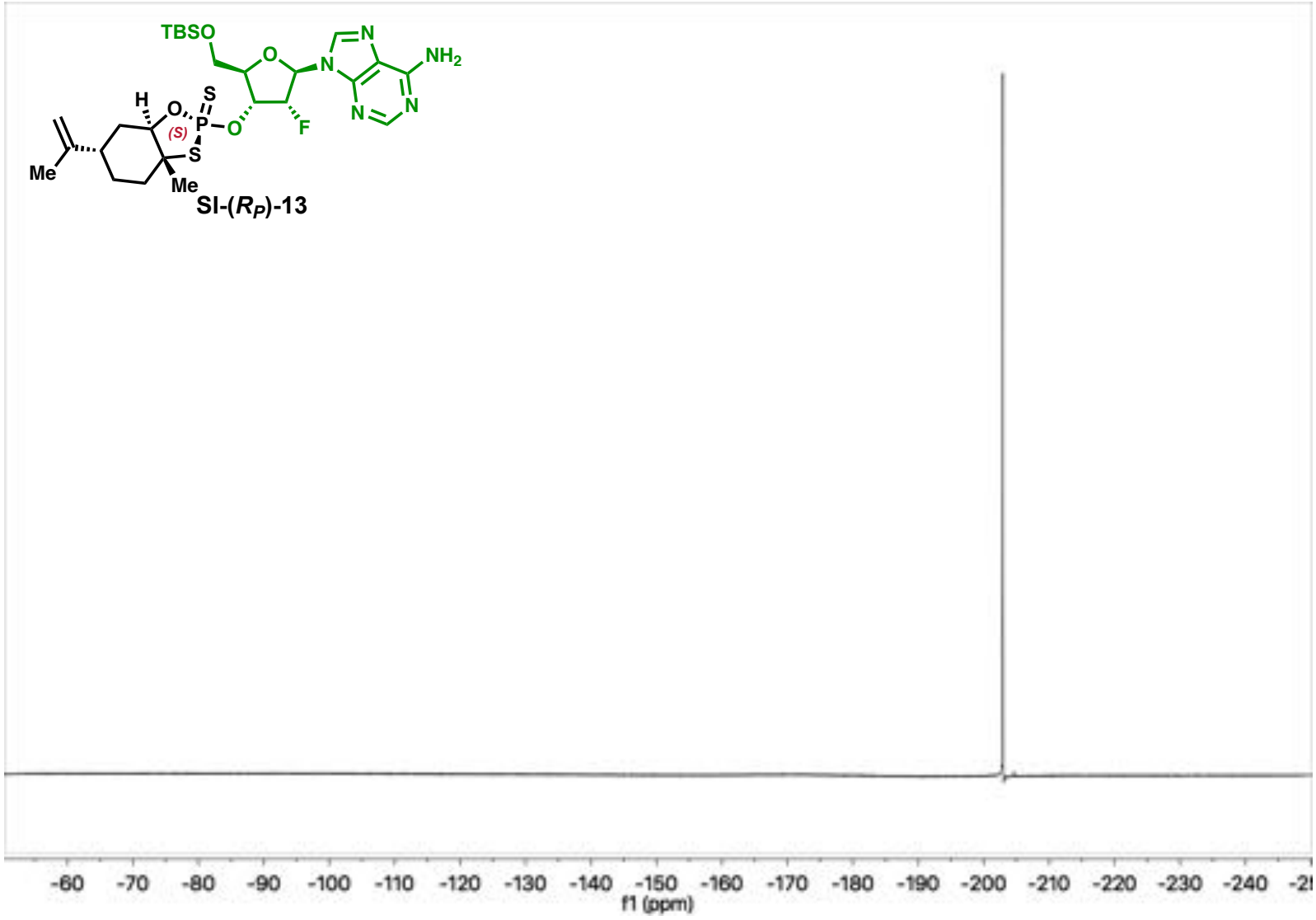
Compound SI-(R_P)-13 ^{13}C NMR



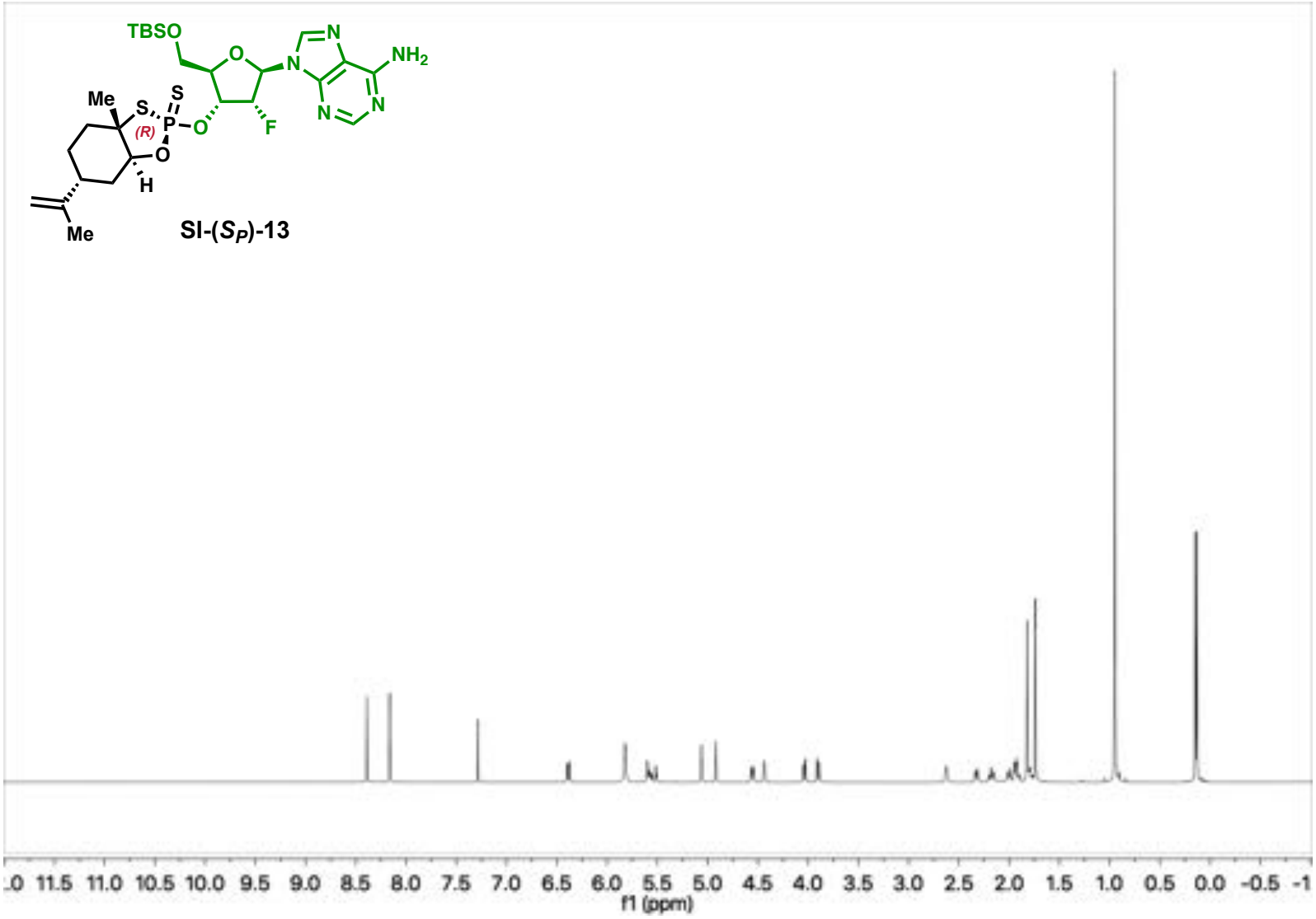
Compound SI-(R_P)-13 ^{31}P NMR



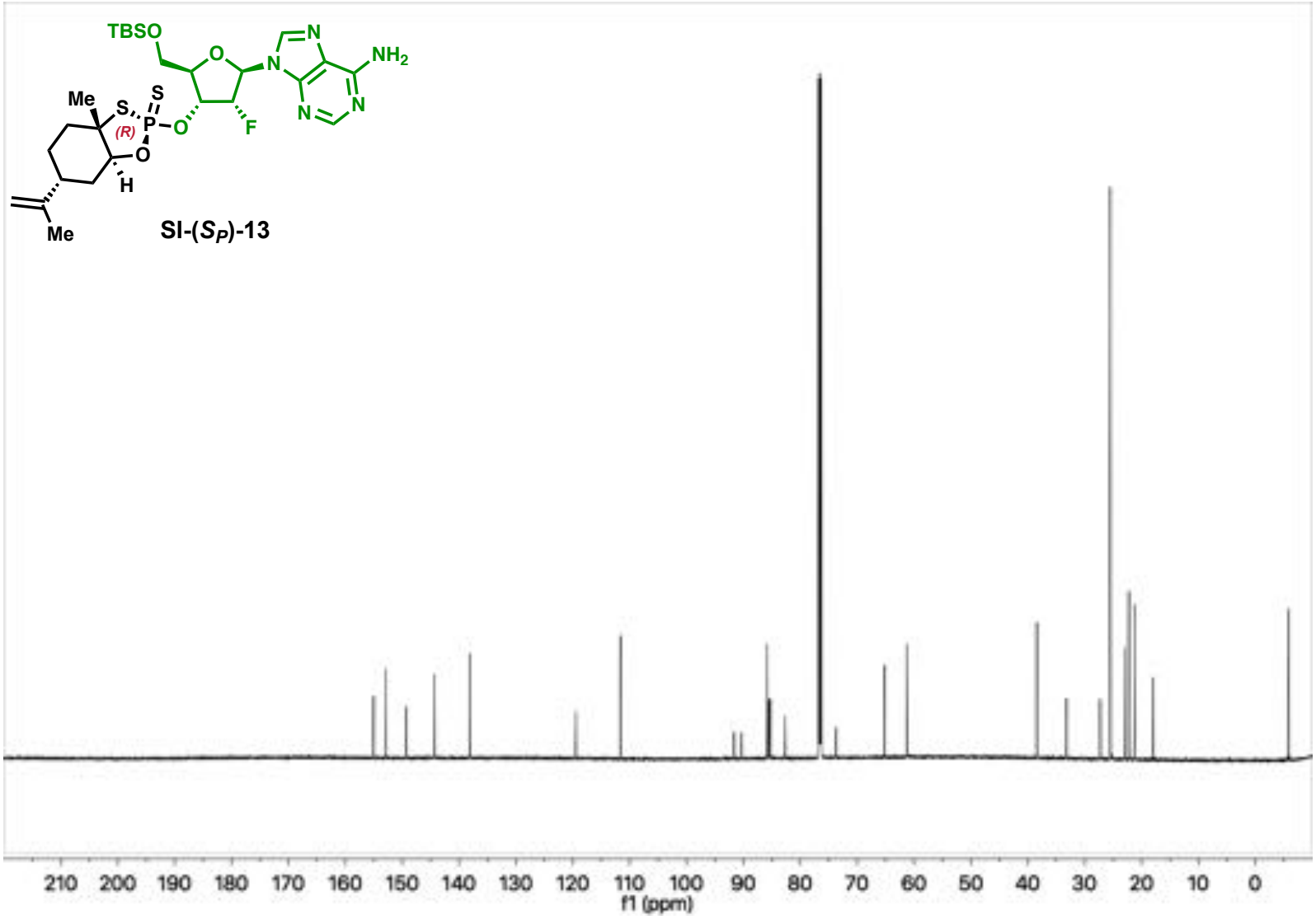
Compound SI-(R_P)-13 ^{19}F NMR



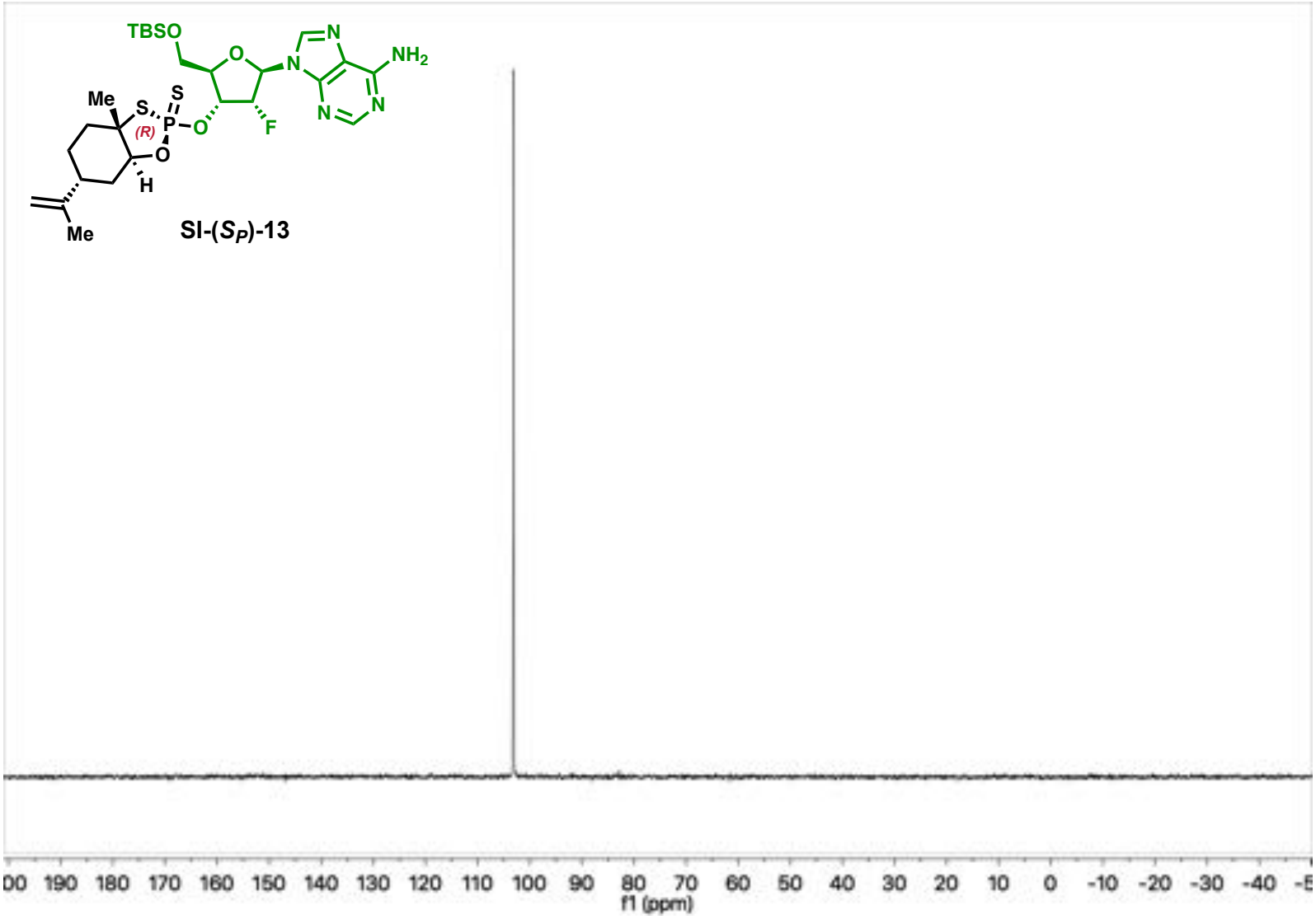
Compound SI-(S_P)-13 ^{1}H NMR



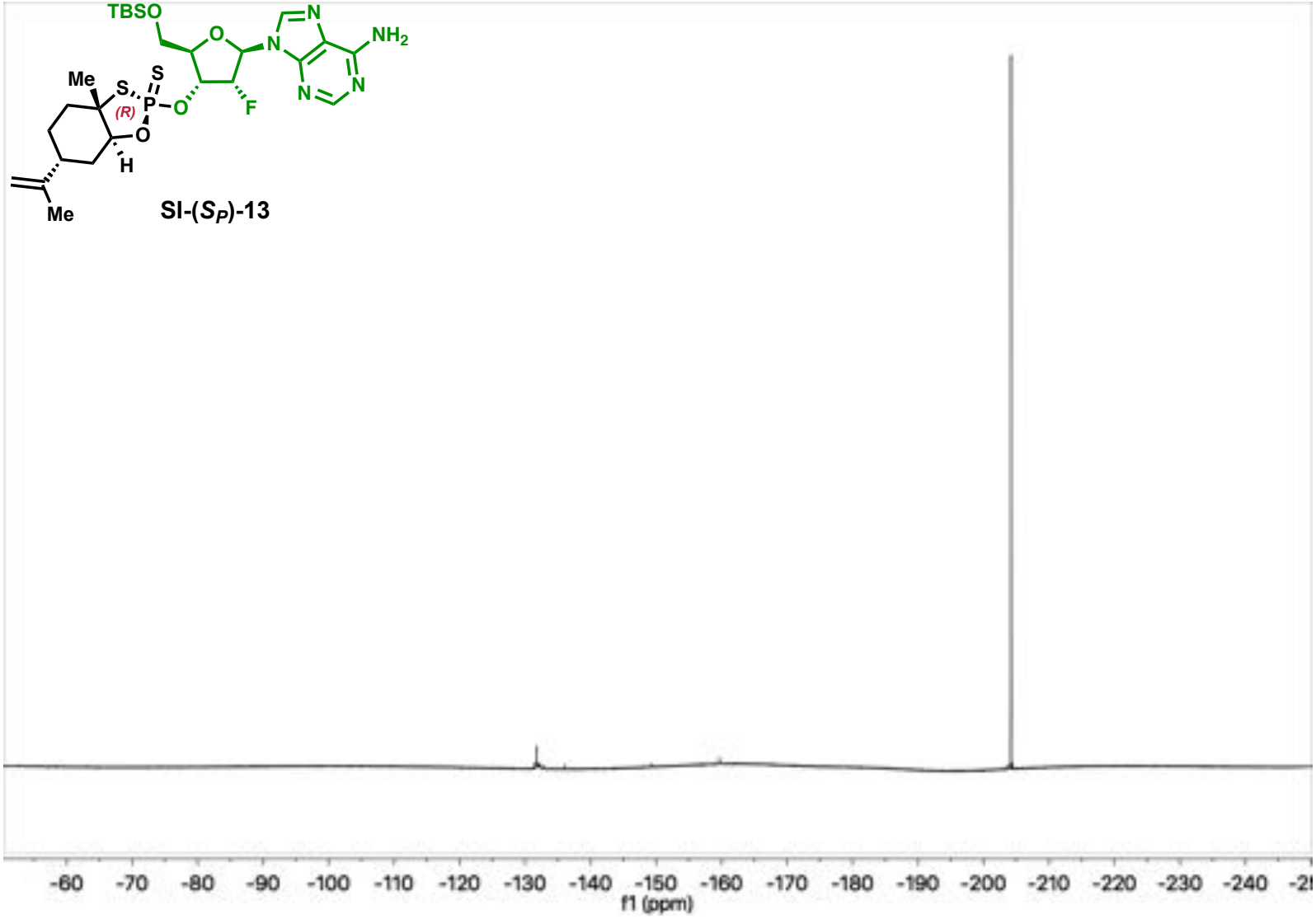
Compound SI-(S_P)-13 ^{13}C NMR



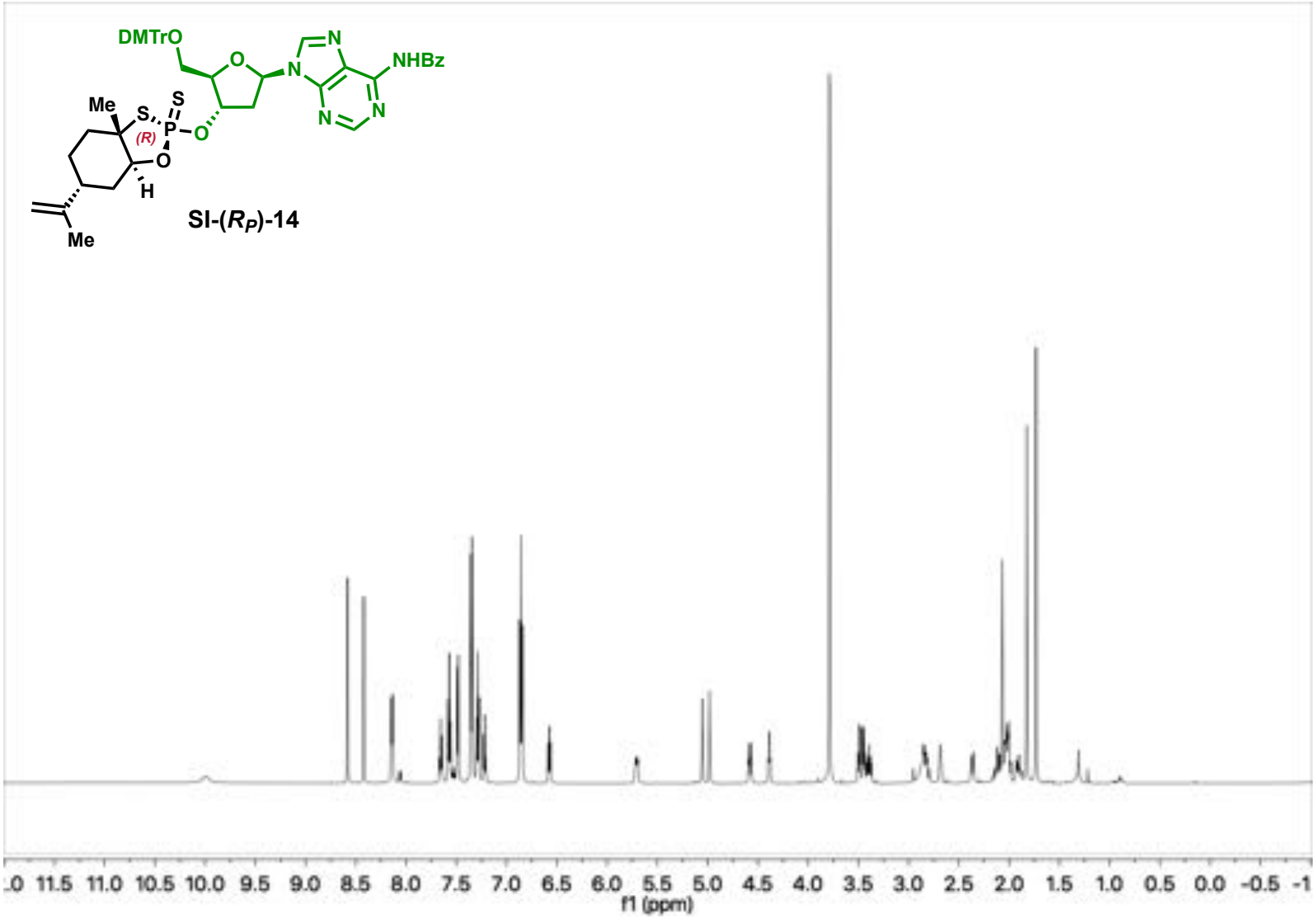
Compound SI-(S_P)-13 ^{31}P NMR



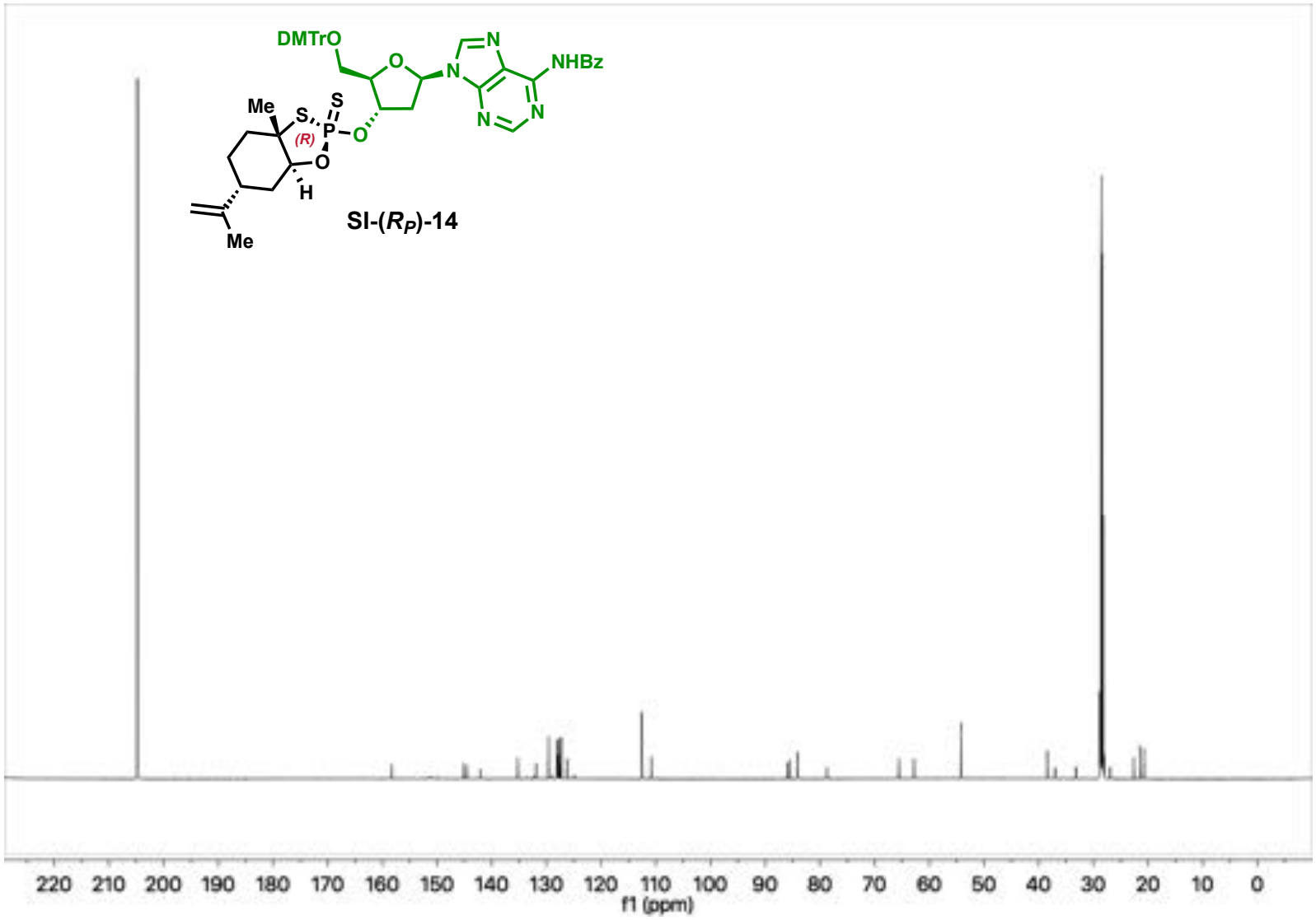
Compound SI-(S_P)-13 ^{19}F NMR



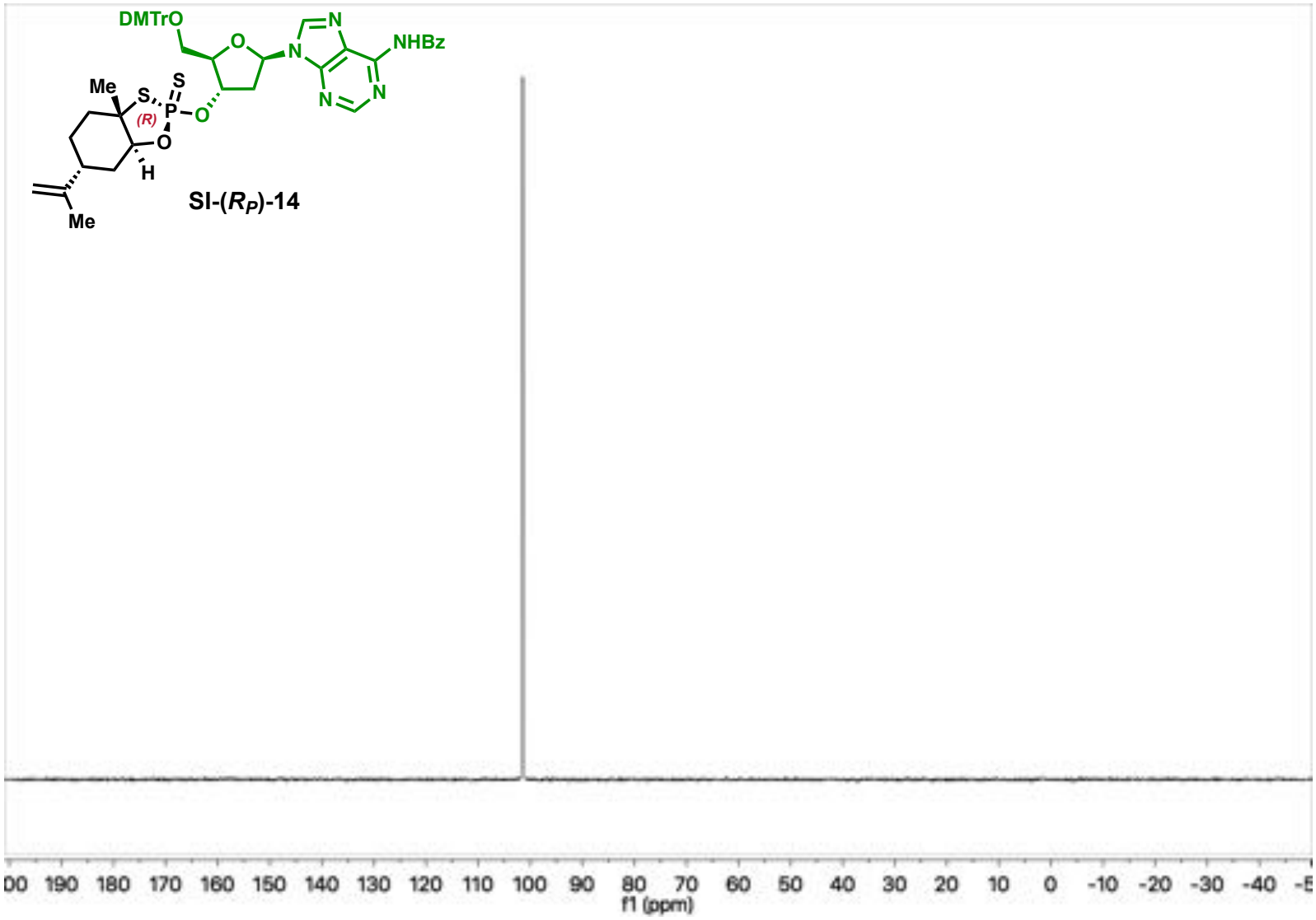
Compound SI-(R_P)-14 ^{1}H NMR



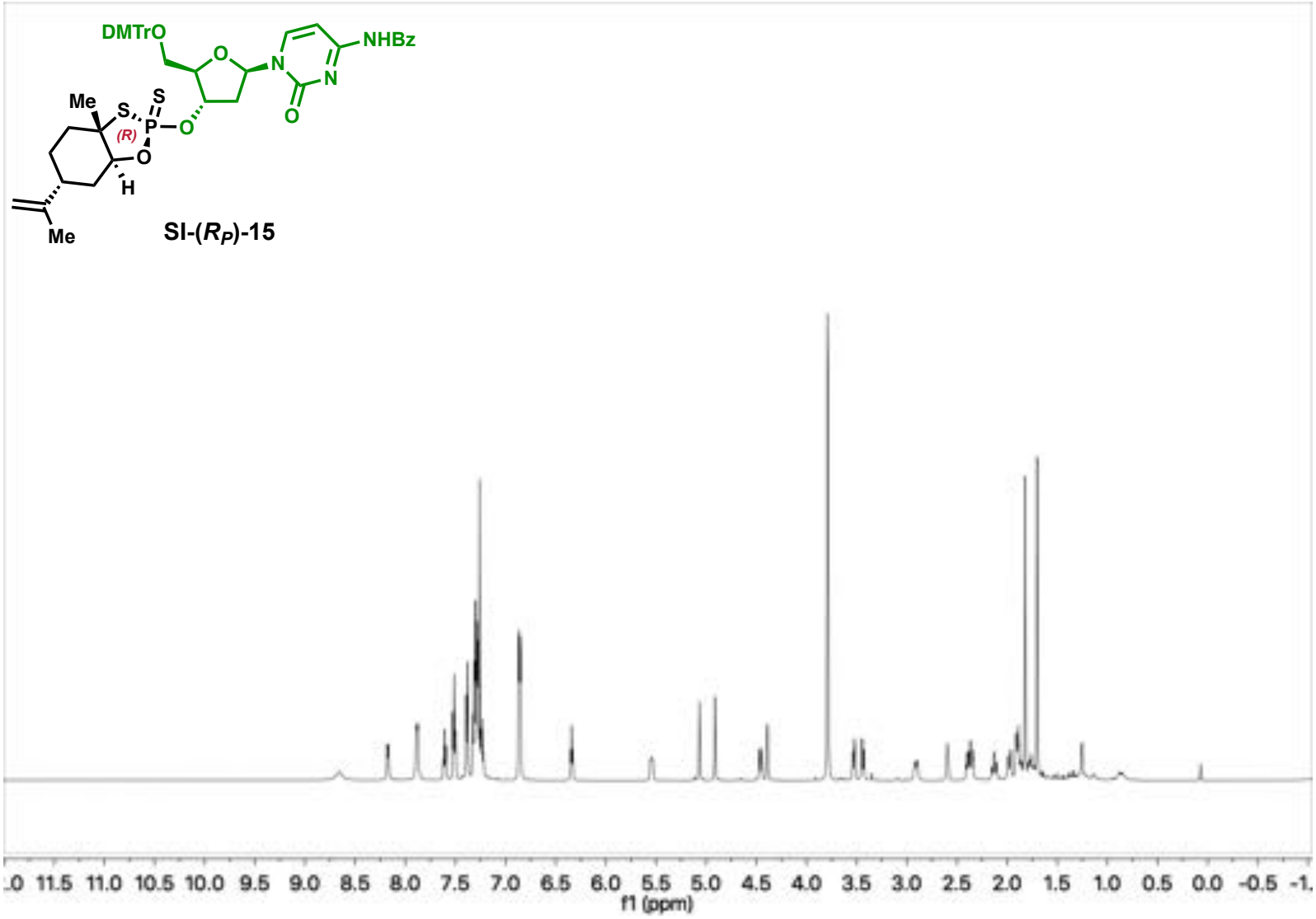
Compound SI-(R_P)-14 ^{13}C NMR



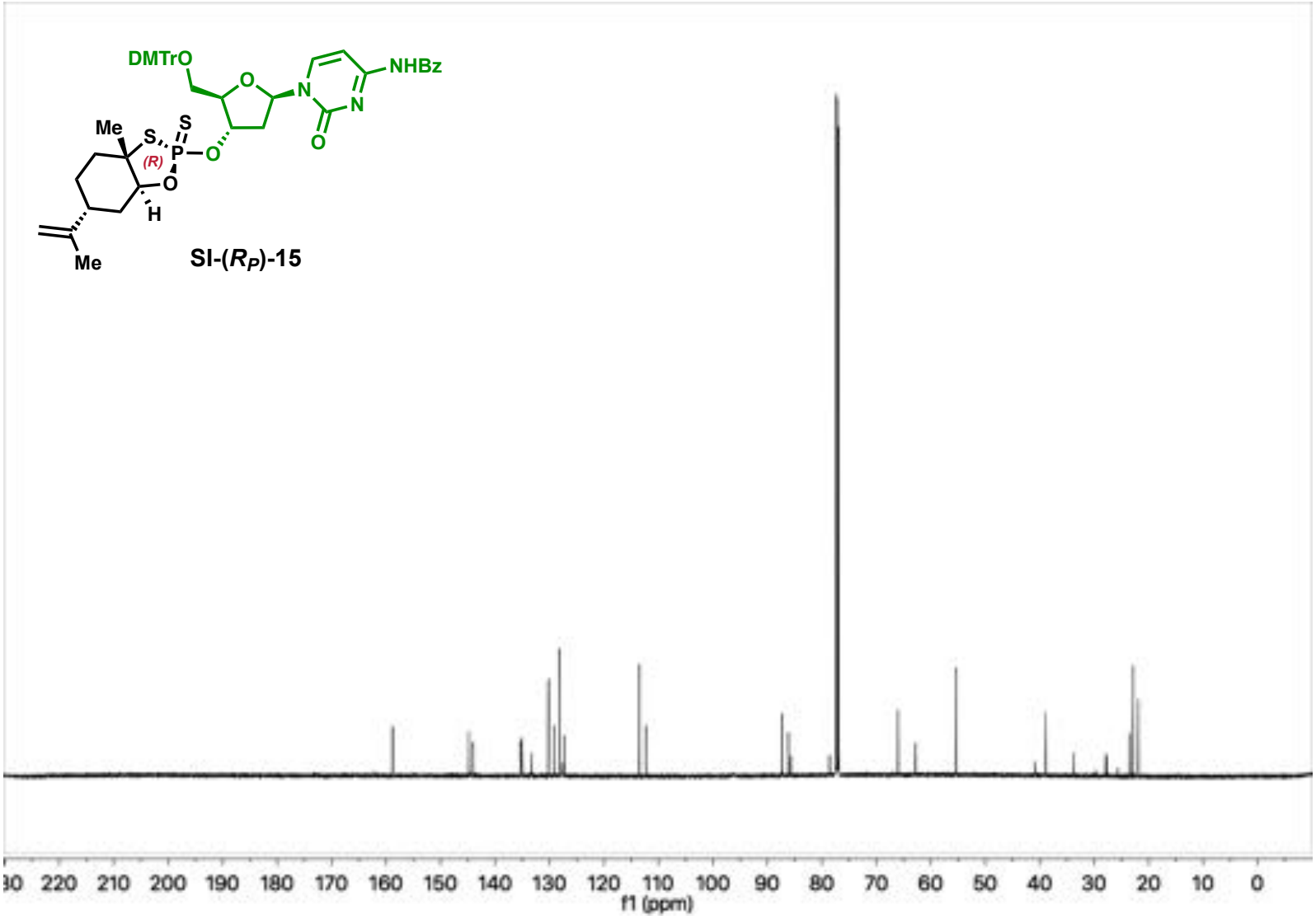
Compound SI-(R_P)-14 ^{31}P NMR



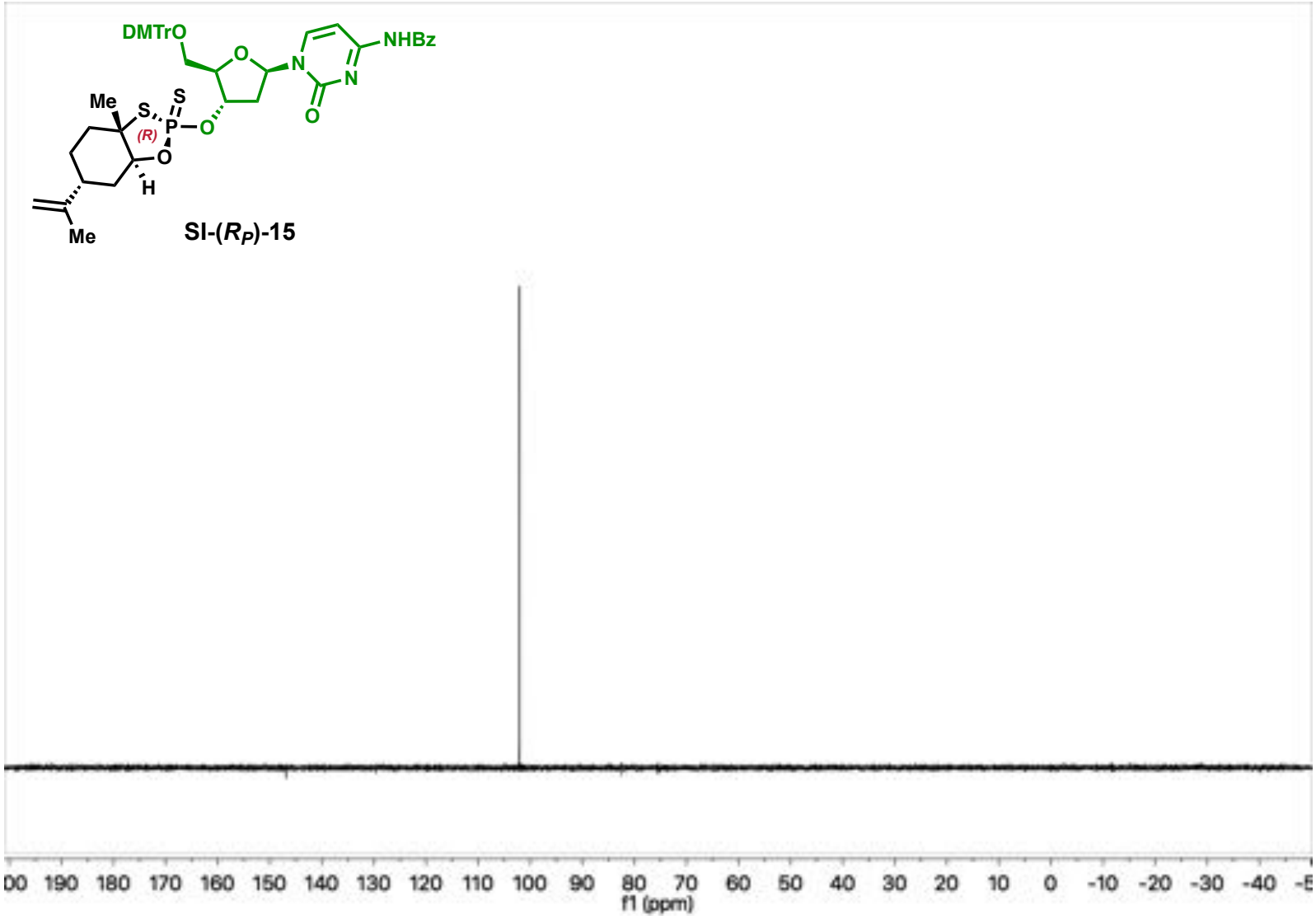
Compound SI-(R_P)-15 ^{1}H NMR



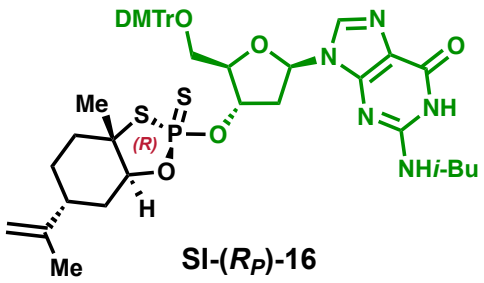
Compound SI-(R_P)-15 ^{13}C NMR



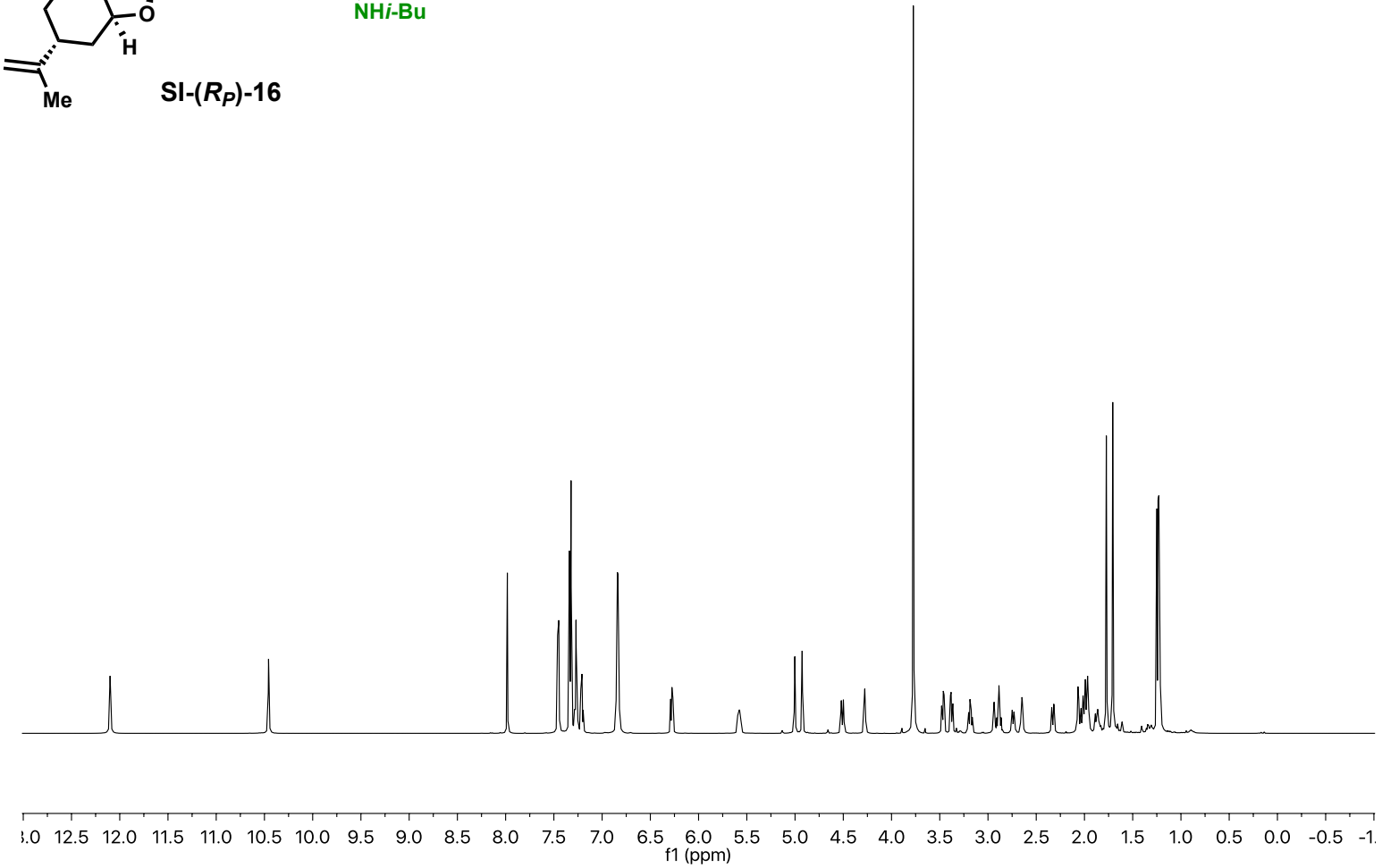
Compound SI-(R_P)-15 ^{31}P NMR



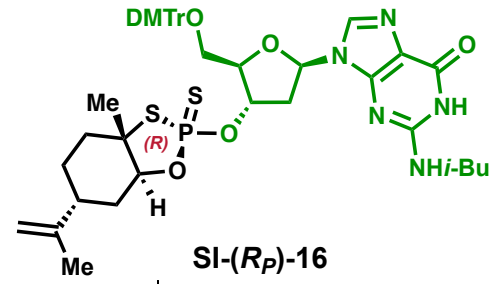
Compound SI-(R_P)-16 ^{1}H NMR



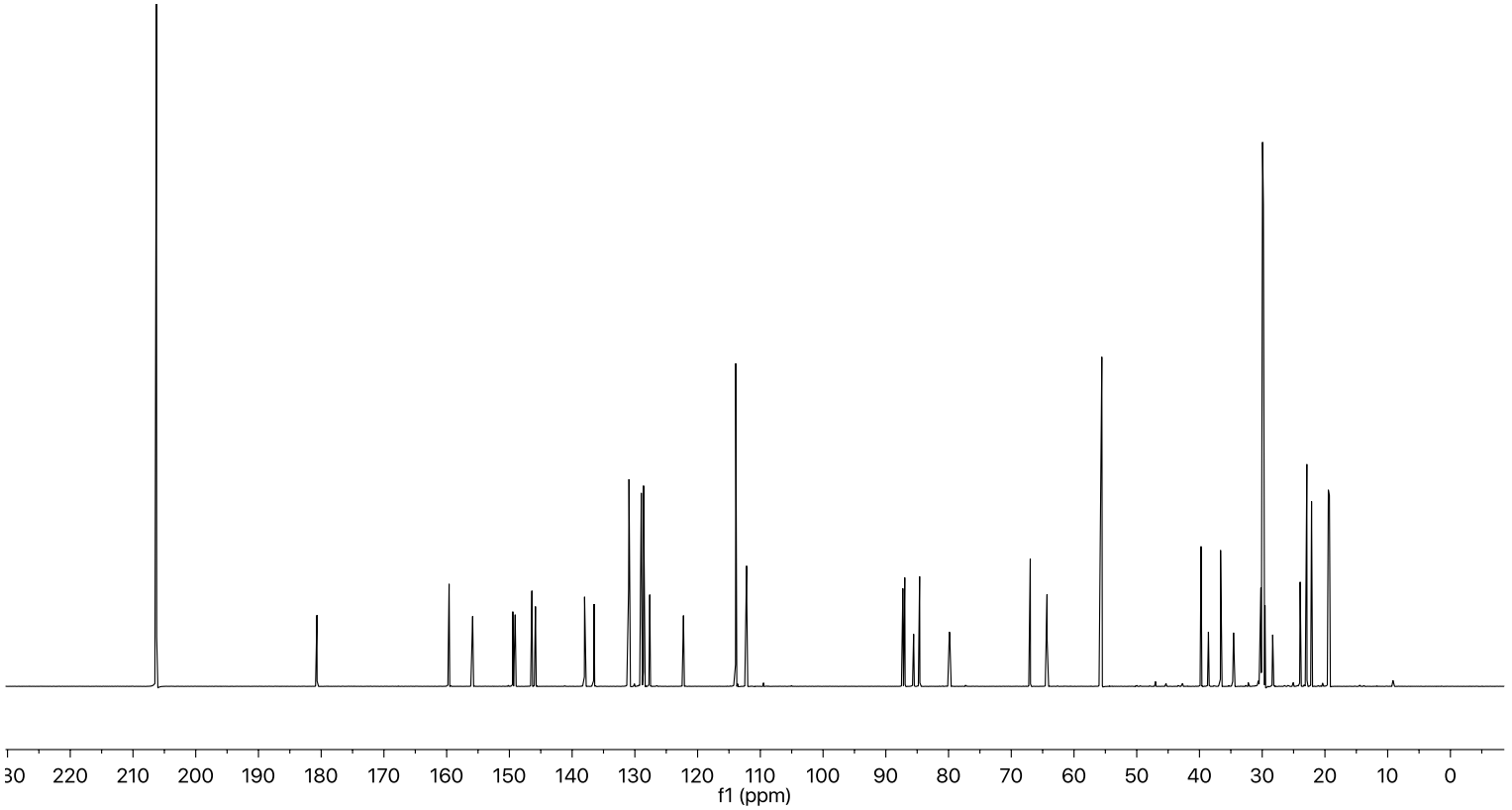
SI-(R_P)-16



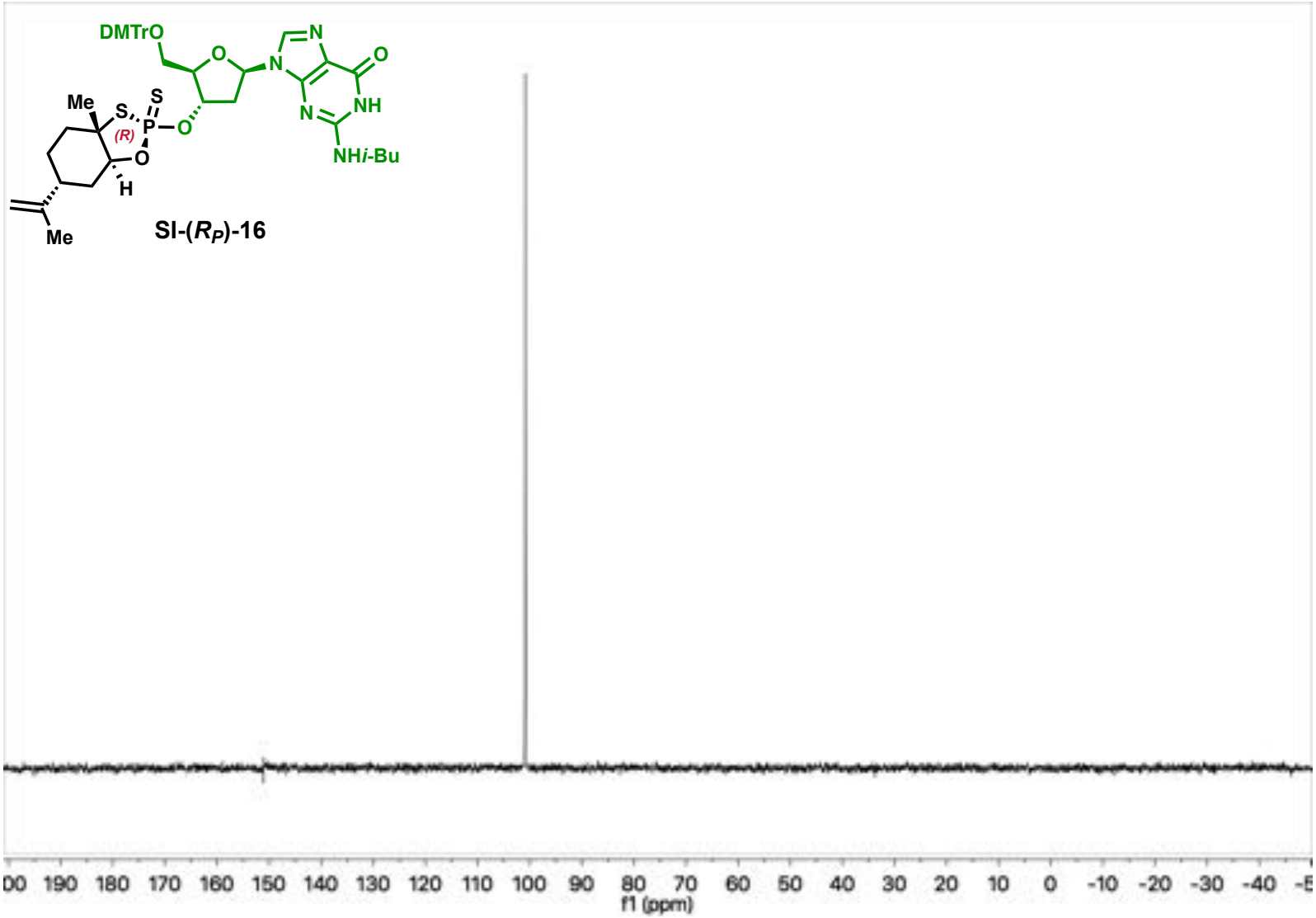
Compound SI-(R_P)-16 ^{13}C NMR



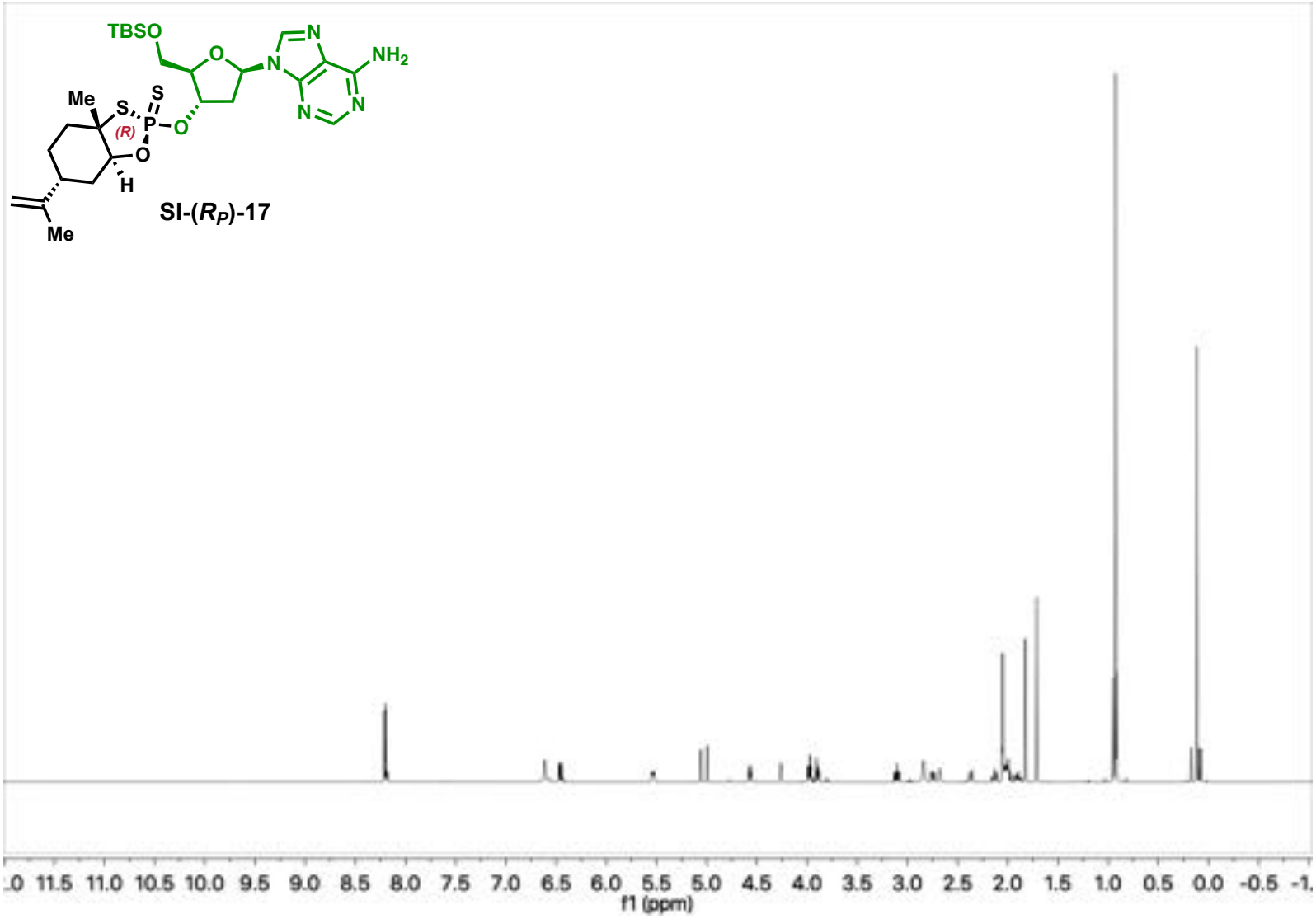
SI-(R_P)-16



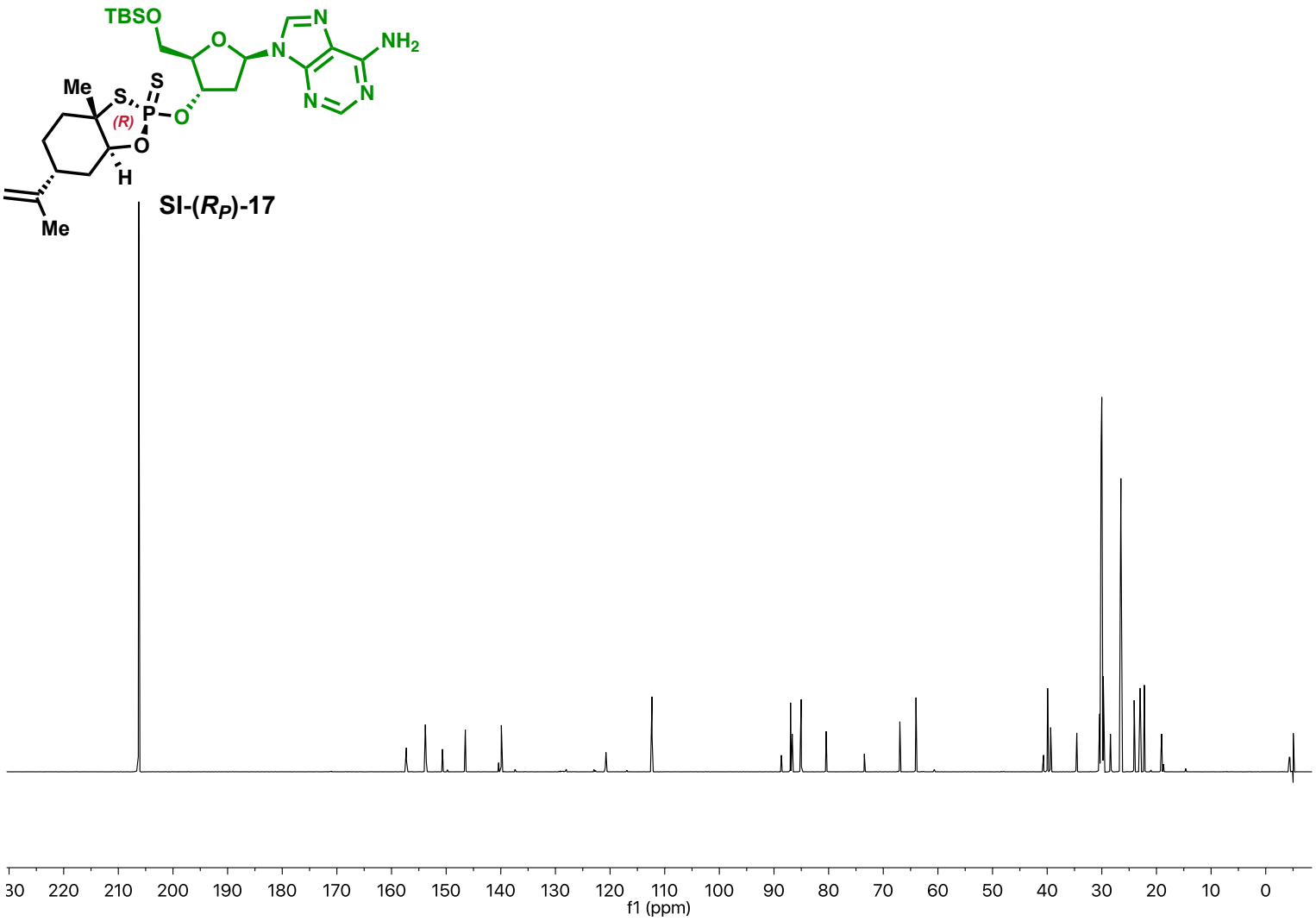
Compound SI-(R_P)-16 ^{31}P NMR



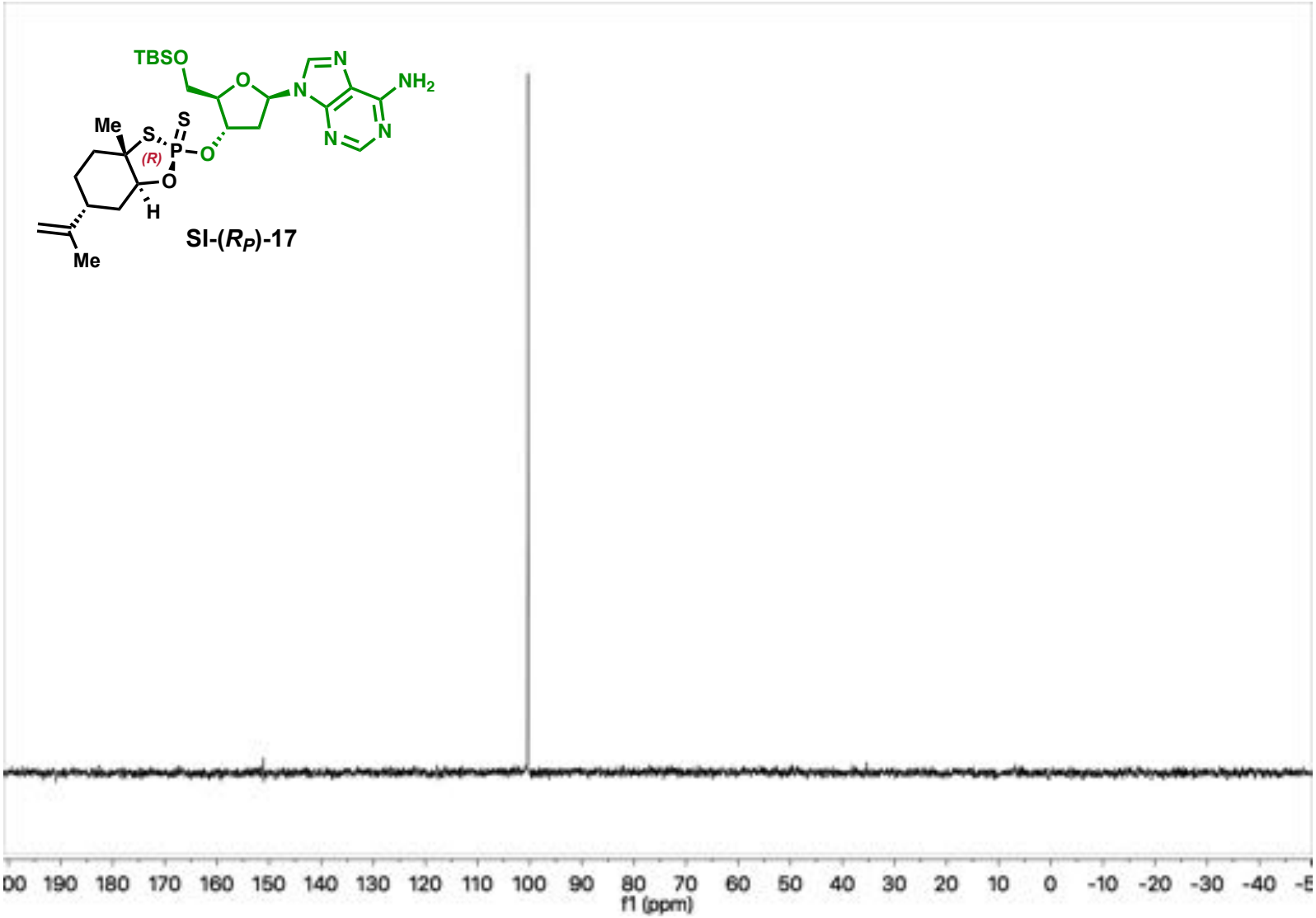
Compound SI-(R_P)-17 ^{1}H NMR



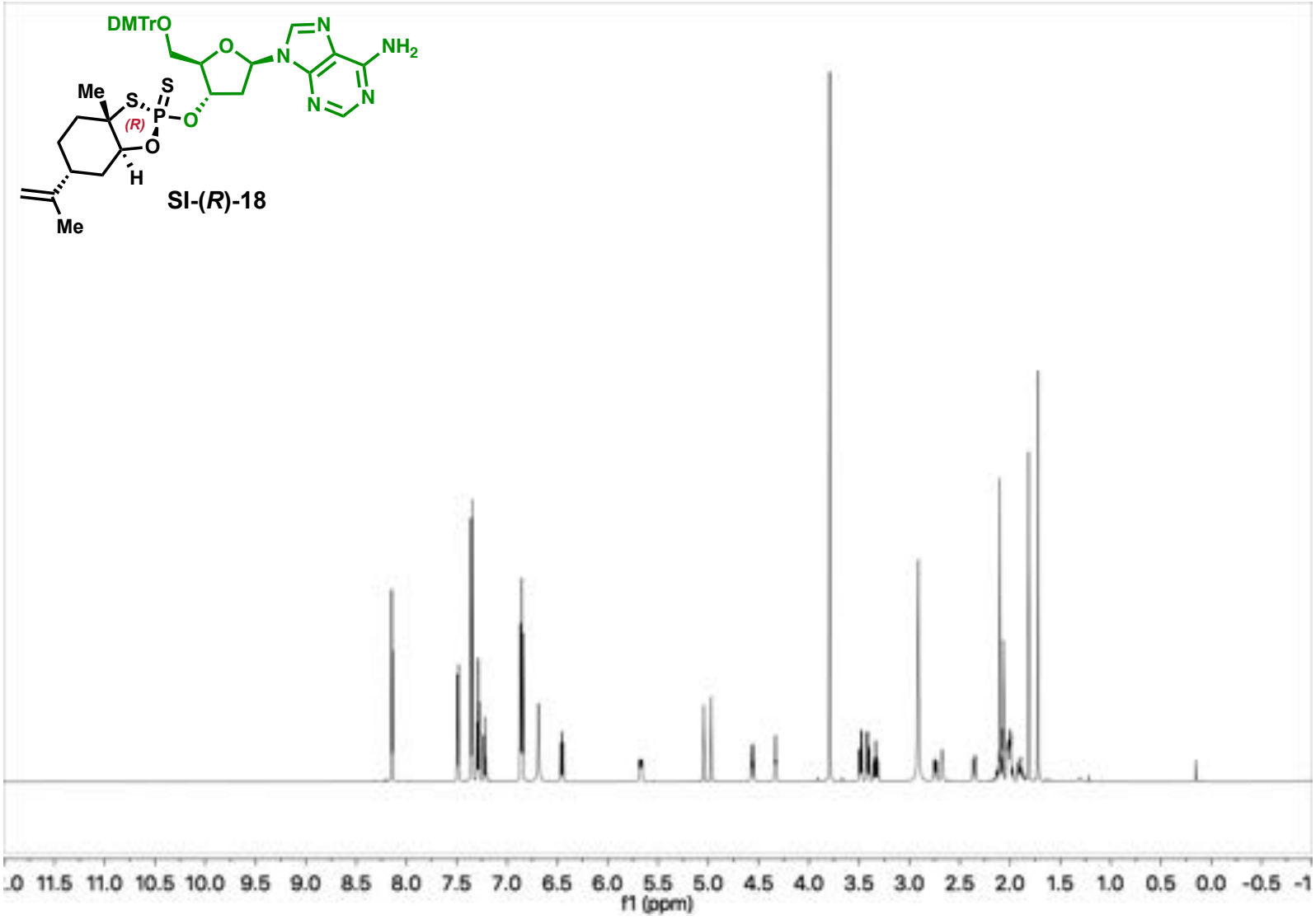
Compound SI-(R_P)-17 ^{13}C NMR



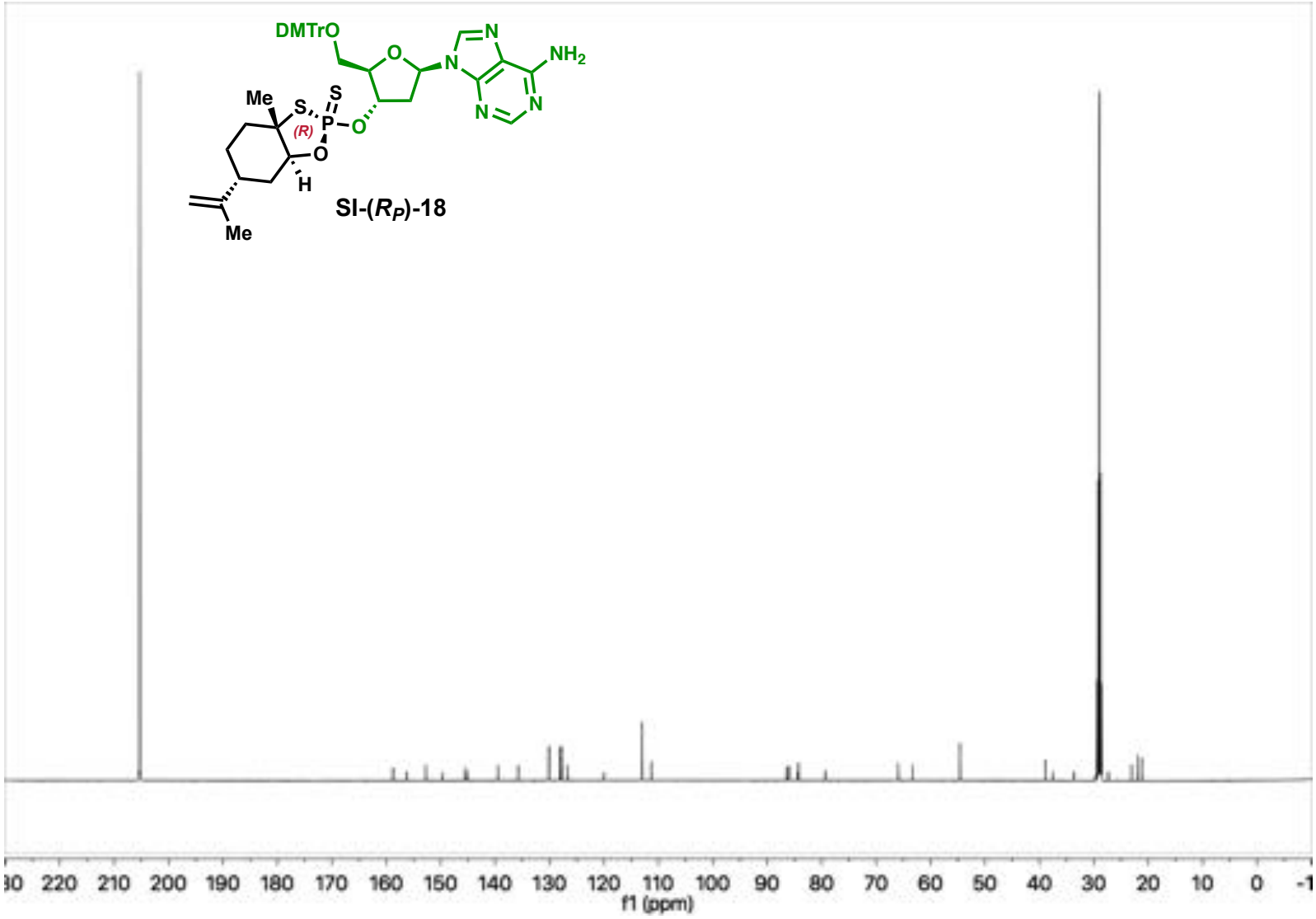
Compound SI-(R_P)-17 ^{31}P NMR



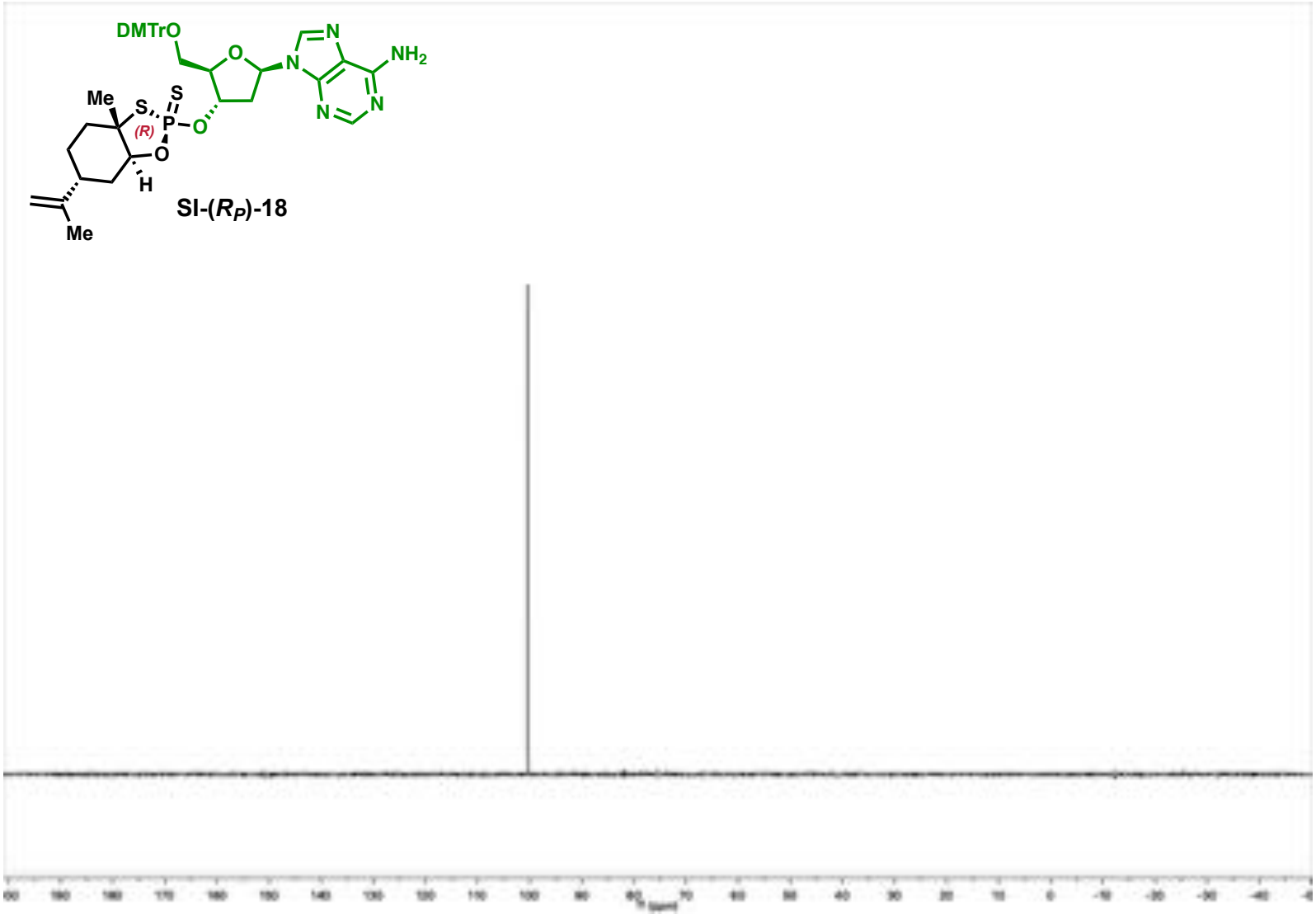
Compound SI-(R_P)-18 ^{1}H NMR



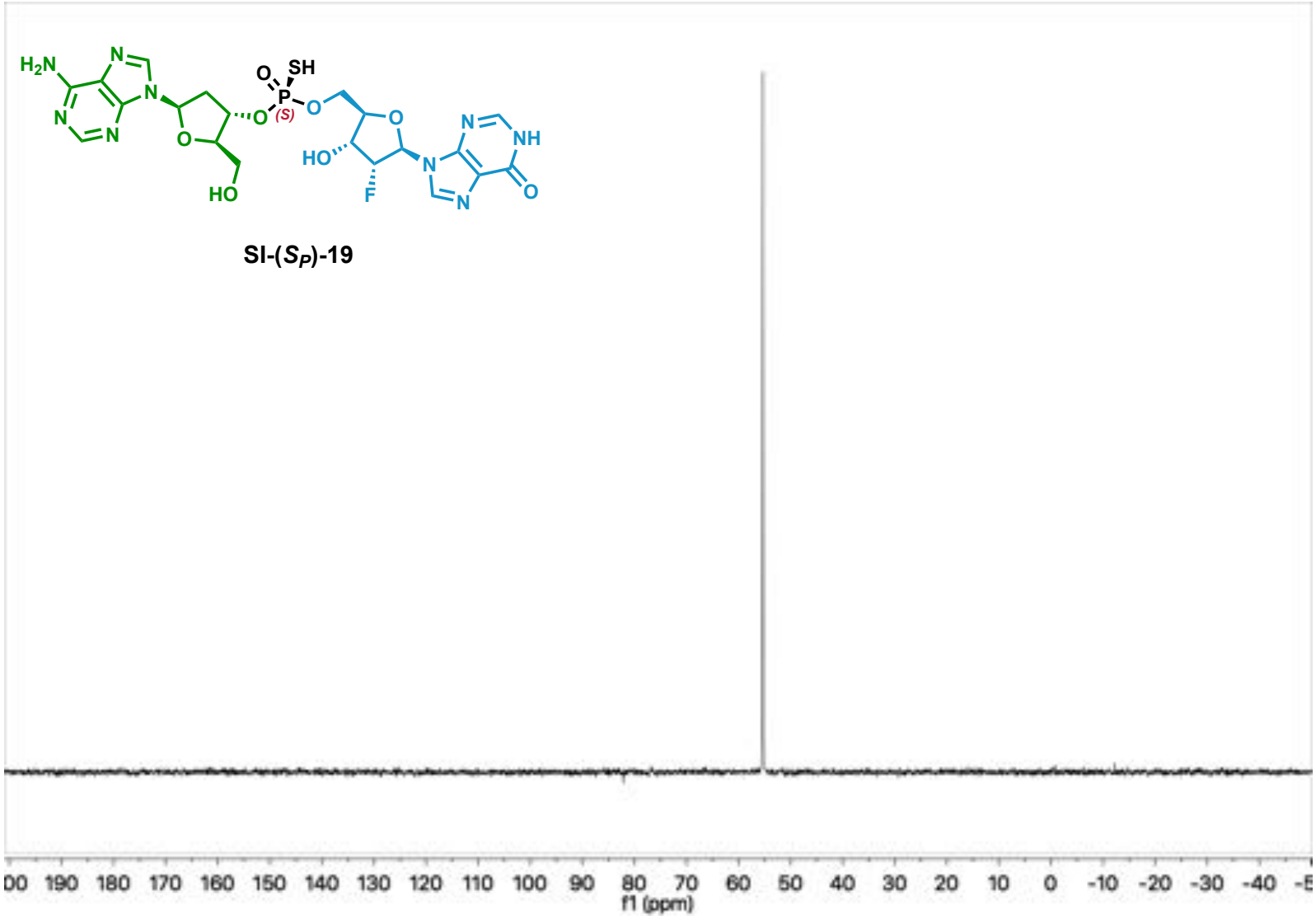
Compound SI-(R_P)-18 ^{13}C NMR



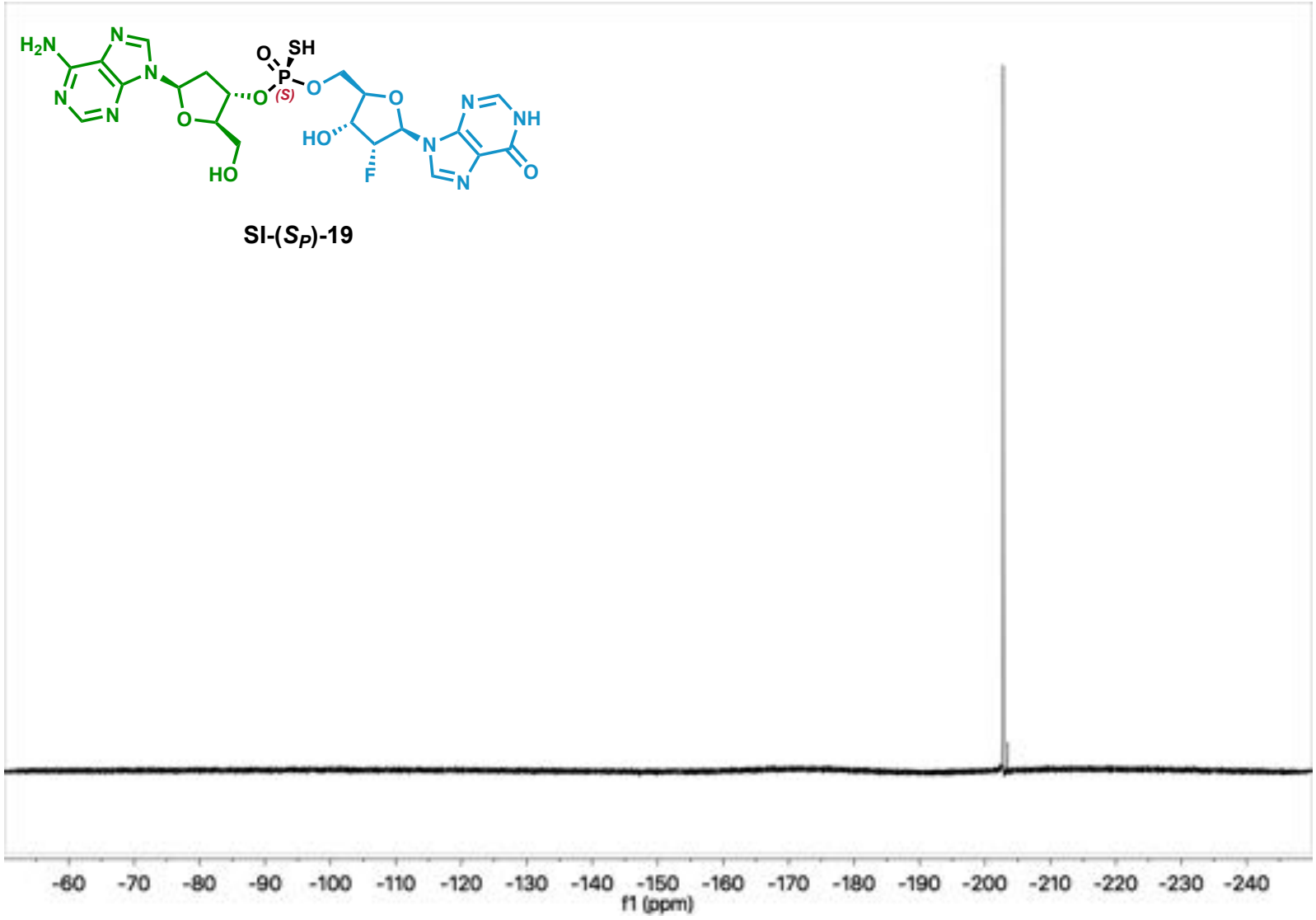
Compound SI-(R_P)-18 ^{31}P NMR



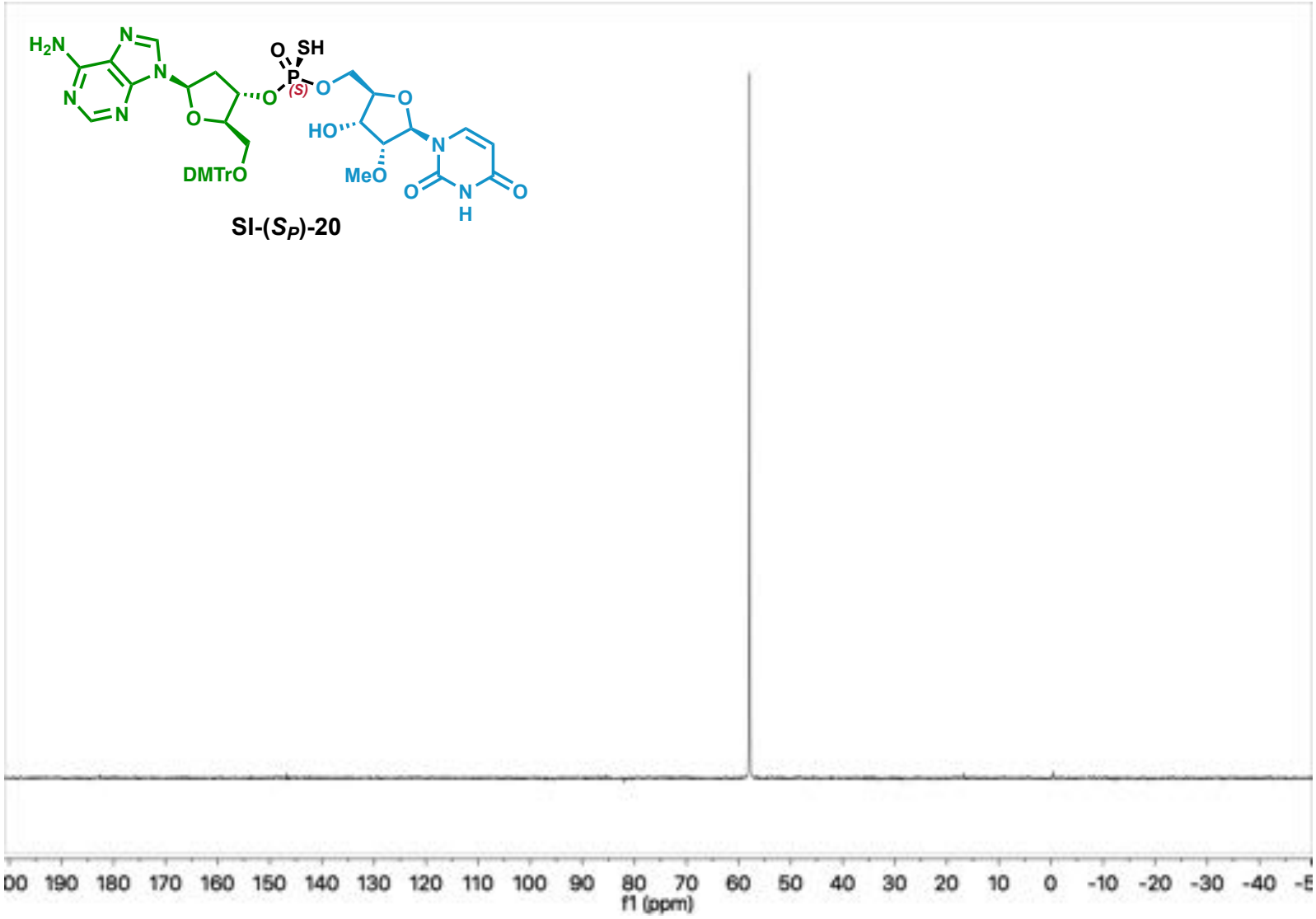
Compound SI-(S_P)-19 ^{31}P NMR



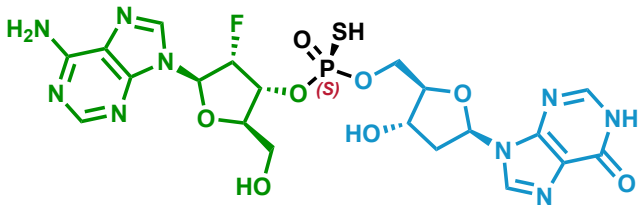
Compound SI-(S_P)-19 ^{19}F NMR



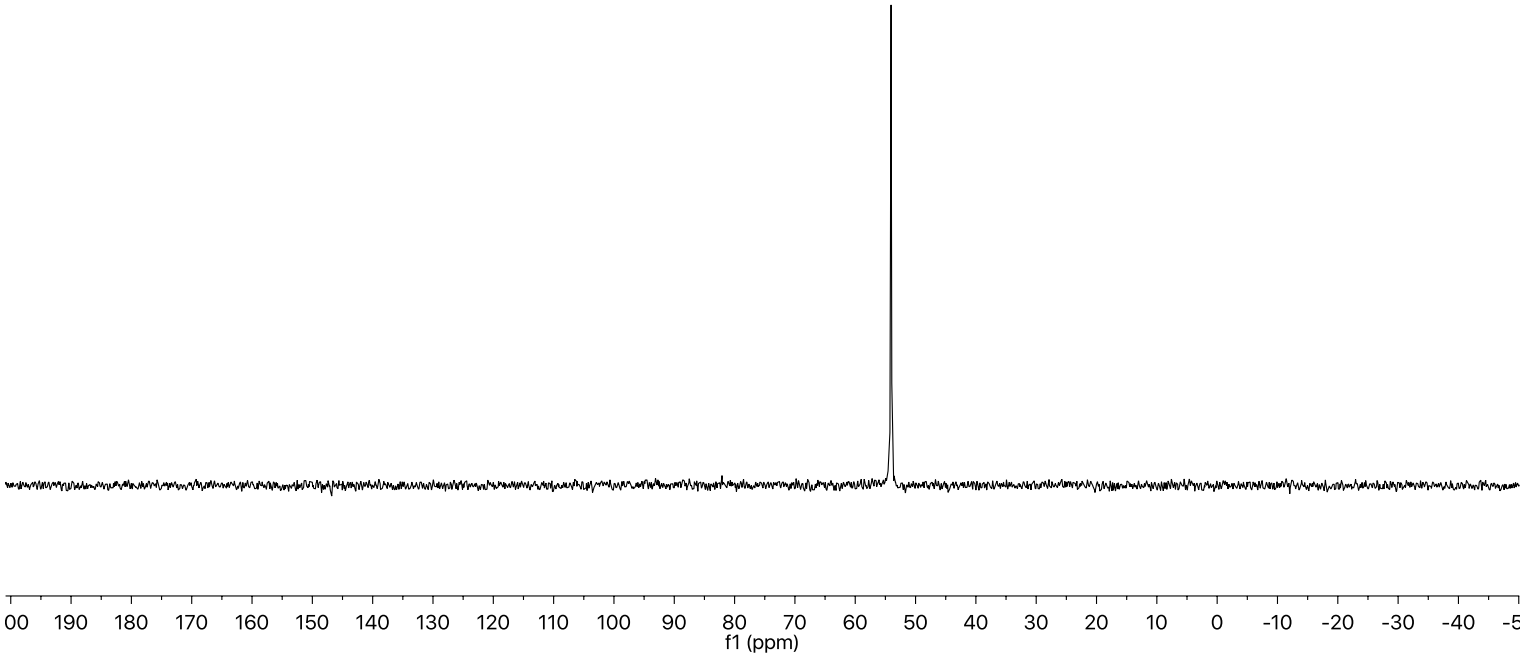
Compound SI-(S_P)-20 ^{31}P NMR



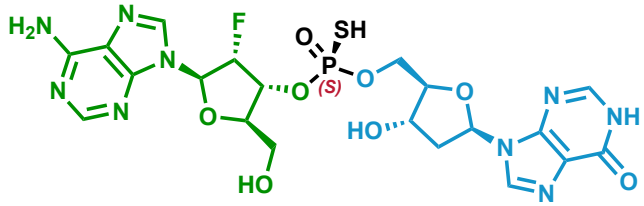
Compound SI-(S_P)-22 ^{31}P NMR



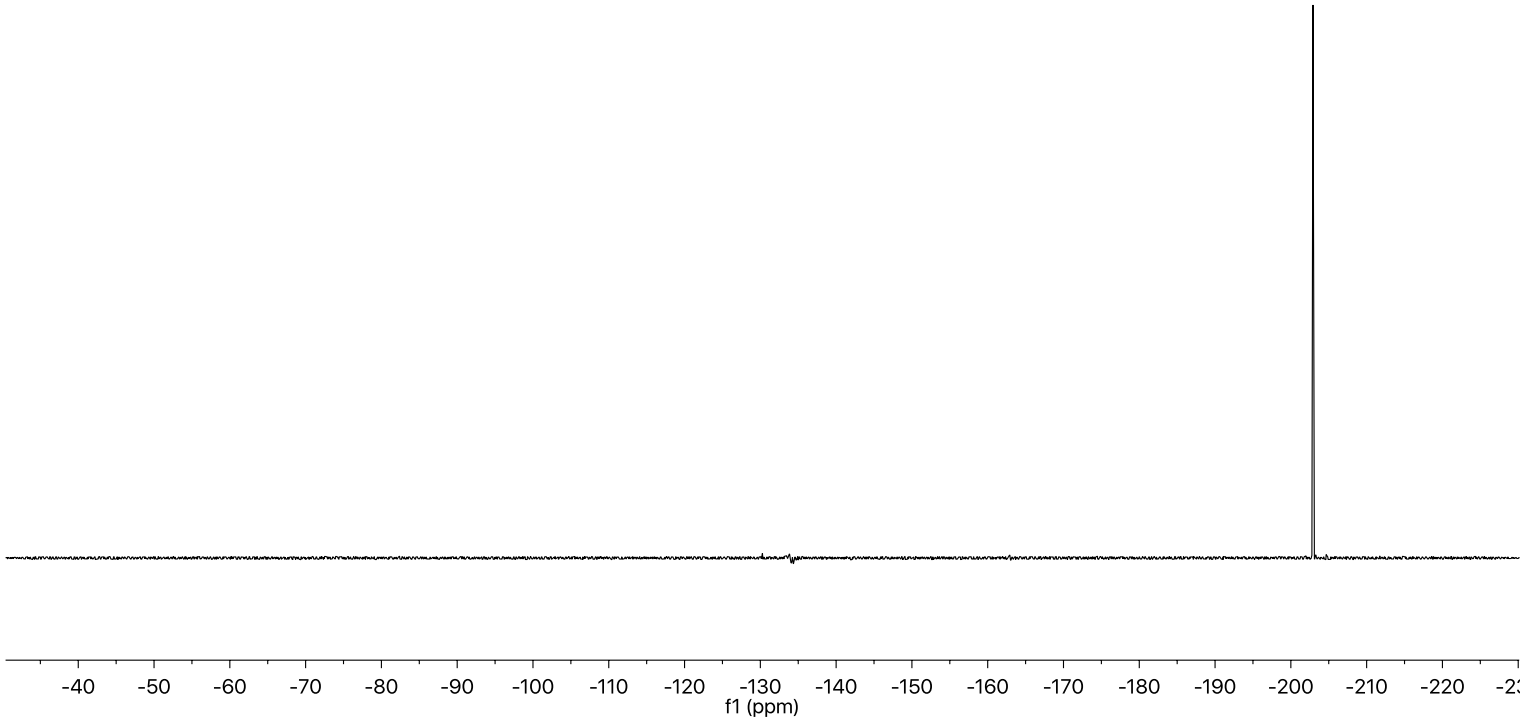
SI-(S_P)-22



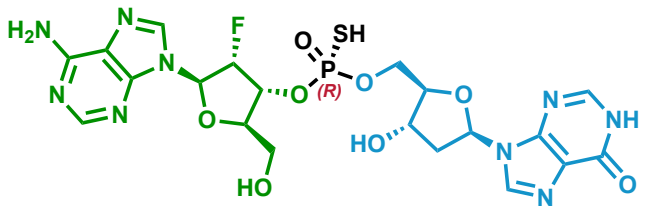
Compound SI-(S_P)-22 ^{19}F NMR



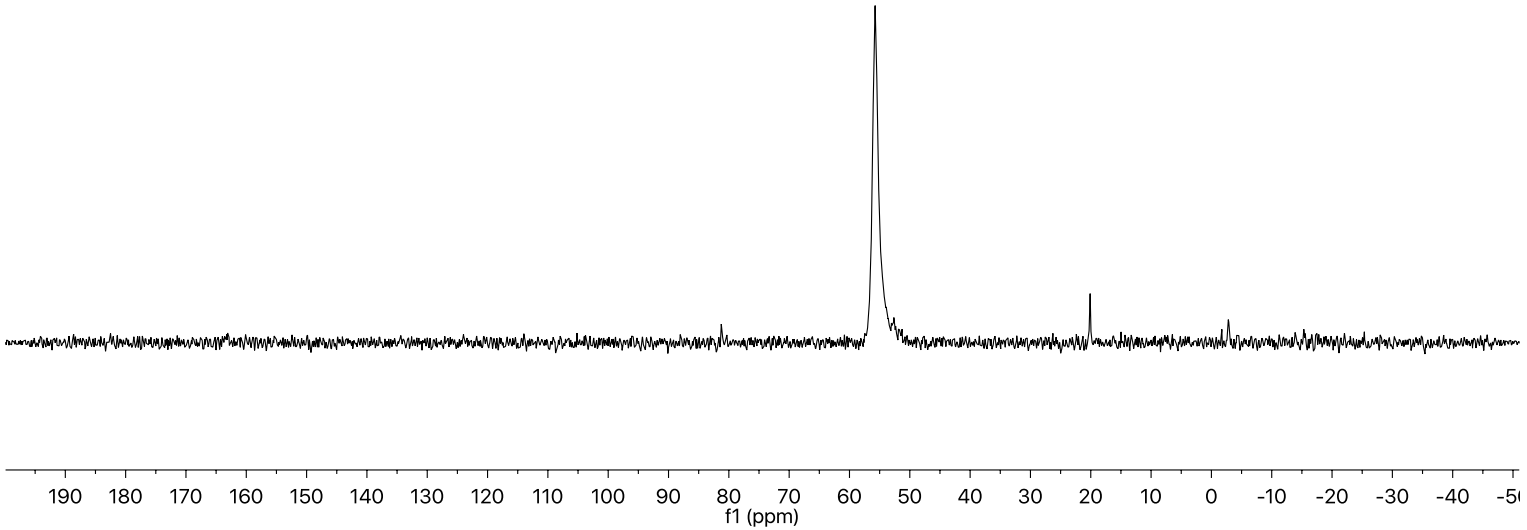
SI-(S_P)-22



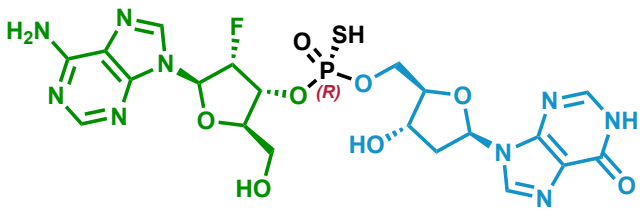
Compound SI-(R_P)-22 ^{31}P NMR



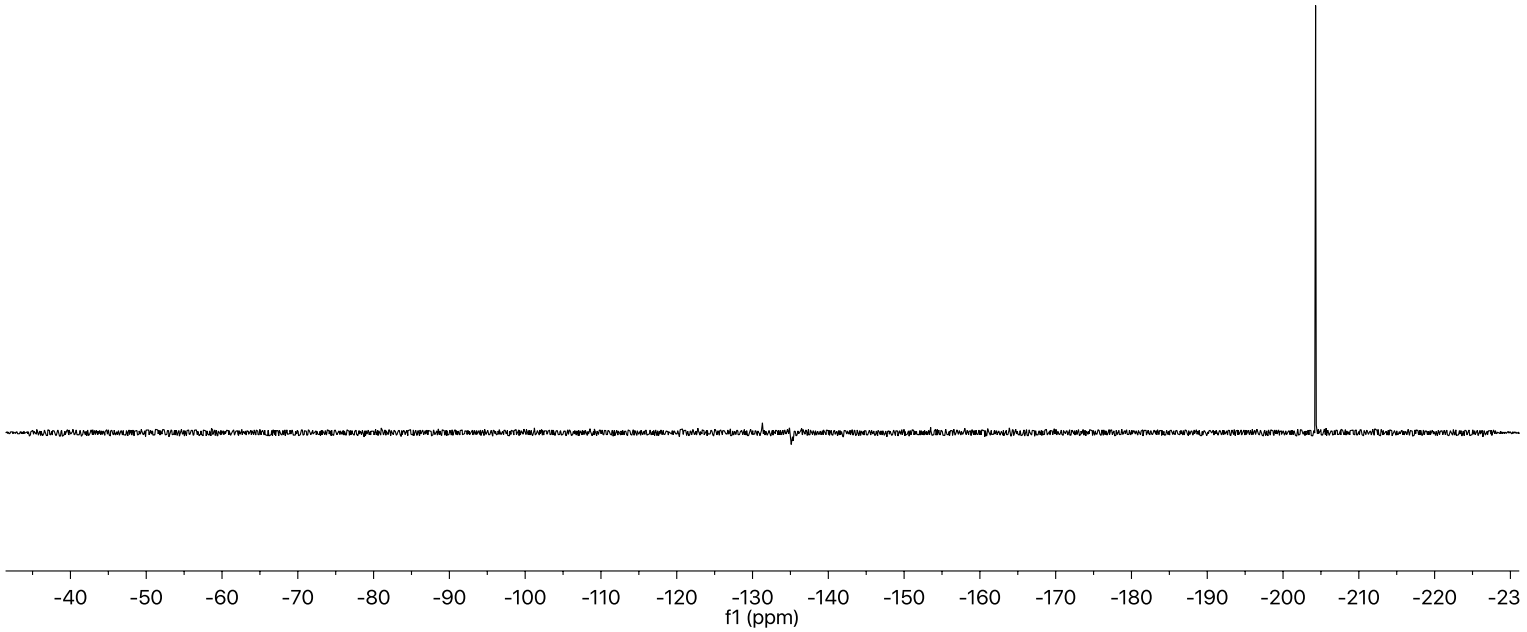
SI-(R_P)-22



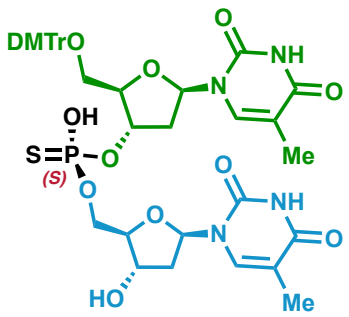
Compound SI-(R_P)-22 ^{19}F NMR



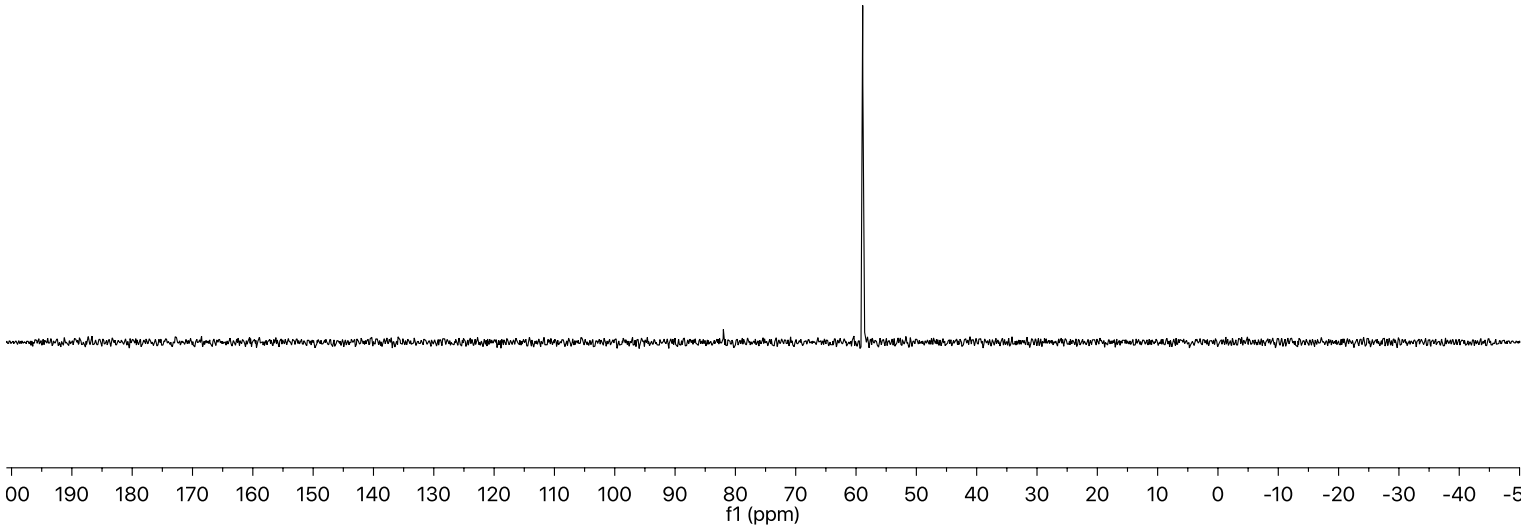
SI-(R_P)-22



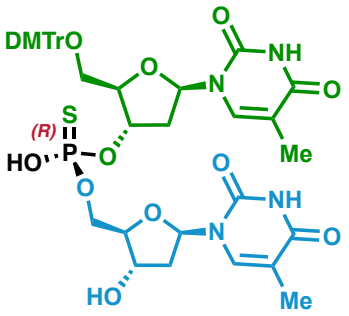
Compound SI-(S_P)-23 ^{31}P NMR



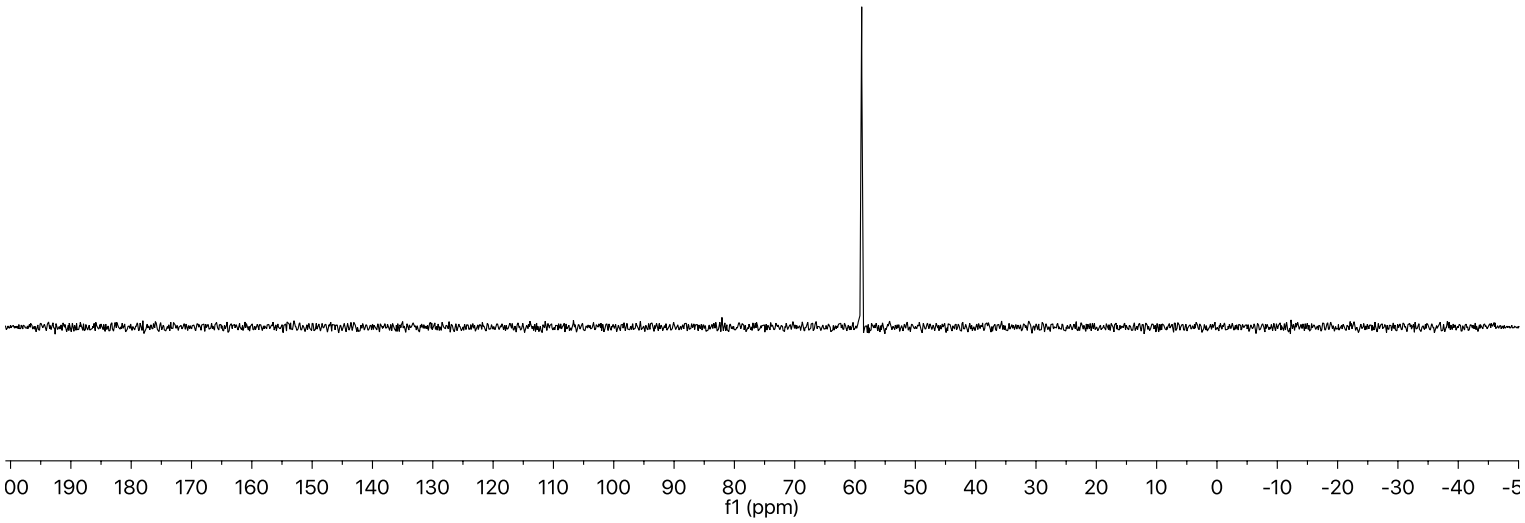
SI-(S_P)-23



Compound SI-(R_P)-23 ^{31}P NMR



SI-(R_P)-23



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